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NOTES AND EXPERIMENTS ON *SARCOCYSTIS TENELLA* RAILLIET

II. SEASONAL INFECTION

JOHN W. SCOTT

In a former paper (1915) the writer stated some evidence favoring Darling's suggestion that Sarcosporidia are merely aberrant varieties of the Neosporidia of certain invertebrates. At the time it was mentioned that experiments were in progress to test the question whether this hypothesis was correct or not. Results so far have shown that the control lambs were not entirely free from infection, so a report on these experiments will be deferred until a later paper. Certain definite data have, however, been obtained with reference to the time of year and other conditions under which infection does and does not occur. The present paper will discuss such data. By using more rigid methods of examination it has been found that apparently 100 per cent. of all adult range sheep are infected with sarcocysts. In the work reported here the lambs examined for sarcocysts were raised under various conditions. Except as otherwise stated the experimental lambs were kept in a dry lot from the time of birth; were supplied with city water which comes from deep springs, and were fed baled native hay kept over from the previous season. Those lambs over which there was no control are referred to as range lambs. By a reference to Table 1 it will be noted that in all groups where grazing was involved 100 per cent. of the lambs became infected. This was true whether the lambs were grazed on the range, in dry or wet pasture, or were fed grass, or flowers, taken from the wet pasture (Groups 1, 2, 4, 5, 6). It is also true that seven out of nine of the control lambs became infected (Group 3). Group 8 was treated as control lambs except that each of the eight lambs used was fed twice weekly a different kind of insect. Six out of eight of these lambs became infected. However, two of these lambs had a very heavy infection, in fact, heavier than any of the groups listed except Group 1, which included the range lambs. A full account and a discussion of the significance of these results will be given at another time. They are mentioned here to show the

different conditions under which the lambs were raised from which some of the important data for this paper was derived, and to indicate that one probably should not expect entire uniformity with respect to the time or degree of infection.

TABLE 1.—TO SHOW INFECTION UNDER DIFFERENT CONDITIONS

Groups	Number in Group	Treatment	Number Infected	Per Cent. Infected
1	5	On range until killed	5	100
2	6	Wet pasture, July 15 to September 23	6	100
3	9	Control. Kept in dry lot; no green food	7	77.7
4	6	Dry lot; grazed twice weekly in lot around pond	6	100
5	2	Dry lot; grazed twice weekly in dry grassy pen near pond	2	100
6	2	Dry lot; fed grass and flowers from wet pasture twice weekly	2	100
7	1	Dry lot; watered twice weekly from pond of Group 4	1	100
8	8	Dry lot; each fed a different kind of insect twice weekly	6	75

Group 1 consisted of early spring lambs; other groups were all May lambs. In all lots ewes ran with their lambs.

Of the lambs grouped in Table 1, three of the range lambs were killed July 13, and the other two six weeks later on August 24. Of the experimental lambs, six were killed October 27; six on November 3; six on November 10; five on November 17; six on the following January 13, and five about two months later, on March 16. In each of these lambs the tip of the heart muscle was saved, fixed in absolute alcohol, embedded, cut in serial sections 16μ in thickness, and stained with Delafield's hematoxylin and eosin. This treatment brings out the sarcocyst in sharp contrast to the muscle tissue in all except the earlier, or Bertram's stages, to find which one needs to use a high power and mechanical stage to avoid overlooking them among the muscle nuclei. The work has involved the preparation of several hundred slides. Altho infection is generalized, the tip of the heart was used for examination, since it is easily localized and conditions are uniform.

The sarcocysts vary considerably in shape, dependent chiefly upon location in the body, and partly upon arrangement of the muscle fibers and connective tissue. For example, in the diaphragm the sarcocysts may be much elongated, and may approximate a spindle shaped form. The typical sarcocyst in the heart, however, is circular in cross section and in longitudinal section is approximately the form of an ellipse. In order to readily obtain a measure of volume it was decided to square the mean diameter and multiply this result by the length of the sarcocyst. This product gives the volume of a rectangular solid just large enough to enclose the sarcocyst measured, and generally is very nearly proportional to the volume. Some sarcocysts were too irregular in shape to obtain an accurate measure of volume, and these were excluded from the calculations. In early stages the length is usually

several times the diameter, but later the diameter increases more rapidly than the length. All measurements were made by means of an eyepiece micrometer, and calculated in micra.

In working over the material to determine whether infection had occurred under the various conditions enumerated above, it was observed that the older the lamb the larger was the sarcocyst; this was to be expected. It was also noticed that in those lambs killed in January and March small sarcocysts appeared to be very scarce or entirely wanting. This seemed to indicate that infection did not occur during the winter and suggested a number of problems. Is infection continuous or discontinuous? Does infection occur only in young lambs? That is, does a range lamb become infected for all time while it is a lamb? Or, is infection seasonal, and does it recur year after year? In an attempt to answer these questions it was decided to take careful measurements of all sarcocysts found in these experimental lambs, and in other lambs and old ewes killed at various seasons of the year.

TABLE 2.—TO SHOW THAT THE AVERAGE SIZE OF THE PARASITES INCREASES WITH THE AGE OF THE LAMB

Group	Date Killed	Lambs Examined	Sarcocysts Measured	Mean Diameter	Mean Length	Mean Volume × Mean D^2 × Mean L.
1	July 13	3	29	29.7	101.7	89,708
2	Aug. 24	2	20	29.6	119.5	104,701
3	Oct. 27–Nov. 17	19	109	30.4	106.3	98,238
4	Jan. 13	6	51	34.5	120.4	143,306
5	Mar. 16	5	39	41.9	110.6	194,170
6	Oct. 27–Nov. 17	3	17	28.3	101.2	81,050
7	Jan. 13	3	25	34.0	125.5	145,078
8	Mar. 16	1	9	48.8	100.3	238,858

Measurements are given in micra. Group numbers do not correspond to groups of Table 1.

A reference to Table 2 shows that the average size of the parasite increases with the age of the lamb. A brief explanation will make this clear. The first column gives group numbers; the second, the dates when the groups were killed; the third column gives the number of lambs examined, and the fourth the number of sarcocysts measured in each group; in the fifth and sixth columns are found the mean diameter and mean length of each group of sarcocysts; in the seventh column is found the mean volume of each group of sarcocysts given in cubic micra; this volume was obtained by squaring the mean diameter and multiplying by the mean length. Groups 1 and 2 were early spring range lambs, the exact age of which is not known; they were, however, as large in August as the experimental lambs were in October, and were probably born in March or early April. Comparing these two groups, killed just six weeks apart, we find the mean volume of the sarcoysts has increased from 89,708 cubic micra on July 13 to 104,701 cubic micra on August 24. Groups 3, 4 and 5 were late April and May lambs; these were all kept in a dry lot until July 15, when

there was begun the experimental work noted above. Groups 4 and 5 were killed at about nine week intervals after Group 3. Here too we find a gradual increase in the mean volume of the parasites. Groups 6, 7 and 8 represent infected control lambs, the data from which are included under Groups 3, 4 and 5, respectively; the data for the control lambs are given separately to show that the same general fact holds in lambs receiving identical treatment and killed at different times.

The fact is also brought out in Table 2 that the mean length, or mean diameter, of a group of sarcocysts is not necessarily proportional to the age of the lamb. In other words, the mean diameter, or mean length, is a variable dependent upon the nature of the tissue in which the sarcocyst is located. The amount of growth increases with the age of the lamb, but the direction of growth is a function of the tissue and depends upon other factors than age. There is also evidence to show that the rate of growth is faster in some lambs than in others; and that sarcocysts are retarded somewhat in growth if they chance to find lodgment in a location unfavorable for nutrition. Since the direction of growth may take place in three directions and since the sarcocyst reproduces by spore formation, it is to be expected, other factors being equal, that the volume of a sarcocyst will increase in a geometrical ratio with arithmetical increments of time. Such data as are available strongly indicate that this conclusion is correct.

That the mean volume of the sarcocysts would increase with the age of the lamb was to be expected. One would anticipate this result whether infection is a continuous or a discontinuous process. Now if infection is continuous, in lambs killed at successive intervals, one may expect the ratio of the volume of the smallest sarcocyst to the volume of the largest sarcocyst to increase gradually with the age of the lamb. But if infection is discontinuous, one may expect this ratio to increase until after the infective period is over and then gradually decrease, until after the time arrives for reinfection.

TABLE 3.—TO SHOW THAT INFECTION IS DISCONTINUOUS

Group	Date	Number Lambs Used	Sarco-cysts Measured	Mean Volume of Parasites	Ratio of Volume Smallest : Largest	Ratio of Volume Smallest : Mean
1	July 13	3	29	89,708	1 : 70.18	1 : 11.95
2	Aug. 24	2	20	104,701	1 : 75.87	1 : 18.21
3	Oct. 27-Nov. 17	19	109	98,238	1 :109.76	1 : 18.60
4	Jan. 13	6	51	143,306	1 : 9.71	1 : 2.43
5	Mar. 16	5	39	194,170	1 : 8.11	1 : 3.77
6	Oct. 27-Nov. 17	3	17	81,050	1 : 21.08	1 : 9.42
7	Jan. 13	3	25	145,078	1 : 3.09	1 : 1.91
8	Mar. 16	1	9	238,858	1 : 6.00	1 : 3.42

Same groups compared as in Table 2. Note that while the mean volume of the sarcocyst increases with the age of the lamb, after a considerable period the ratio of the smallest to the largest parasite decreases instead of continuing to increase.

A study of Table 3 shows that this in general is what one finds. The ratio of the smallest to the largest of twenty-nine sarcocysts killed on July 13 was 1 to 70.18. By August 24, of twenty sarcocysts measured, the ratio had increased to 1 to 75.87, thus indicating continuous infection. These ratios probably do not give the best sort of a comparison, for if one leaves out of consideration the largest parasite for each date, the ratios become, on July 13, 1 to 35.18 and on August 24, 1 to 57.79. It should be mentioned that the smallest parasites found for these dates had about the same volume. Or, if one takes the ratio of the smallest parasite to the mean volume, it becomes for July 13, 1 to 11.95, and for August 24, 1 to 18.21. In any case both the ratios and the mean volume increase, while the size of the smallest parasite found does not increase. Consequently, infection has been continuous throughout this period.

Now in the experimental lambs, killed October 27 to November 17, the smallest sarcocysts found were about the same size as the smallest found during the summer, but the ratio of the smallest to the largest at this date is 1 to 109.76, and the ratio of the smallest to the mean is 1 to 18.60. Comparing these smallest stages found with the stages figured by Erdmann (1910), it is probable that they represent an age 5 to 7 weeks after infection, and from 1 to 3 weeks after invasion of the musculature. It is probable therefore that infection occurred in these lambs up to about October 1. It is also interesting to make a comparison with the results obtained by killing lambs on January 13 and March 16. The smallest parasite found on these dates had volumes between 50,000 and 60,000 cubic micra, while the smallest parasites found during the summer and fall were only one-ninth or one-tenth as large. On January 13 the ratio of the smallest to the largest parasite was 1 to 9.71, and its ratio to the mean was 1 to 2.43. So one finds that a large decrease in the ratio has occurred in spite of a large increase in volume, and this decrease is due to the fact that small sarcocysts are no longer found. The same holds true on March 16. Here the ratio of the smallest sarcocyst to the largest is 1 to 8.11; that is, the ratio is still decreasing. The ratio of the smallest to the mean volume is 1 to 3.77. This is somewhat larger than the corresponding ratio for January 13, and is accounted for by the fact that the smallest parasite found in these lambs on March 16 had about the same volume as the smallest one found on January 13. A larger series of measurements for these dates would no doubt render more uniform results. I have proof to show that this would be true. In an old ewe killed December 28 the smallest sarcocyst had a volume of 38,304 cubic micra. Considering the rate of growth, one would expect the smallest parasite found on January 13 to have a volume between 40,000 and 50,000 cubic micra, instead of 58,000 as was the case in the few experimental lambs killed on this date. Groups 6, 7 and 8 represent the

results from the control lambs, given separately. Here too the results, to a certain extent, lack uniformity, due to the small number of parasites involved. However, in all cases *it is clear that infection has been discontinuous,* and probably there was *no infection after about the first of October.*

Another question, with reference to how early in the spring does infection begin, has not been so accurately determined. If all sarcocysts had the same rate of growth, one could measure the largest sarcocysts found in lambs at different dates during the summer, and could determine fairly accurately the approximate time when infection begins in the spring. The rate of growth, however, of a particular sarcocyst appears to depend to a considerable extent upon the amount of nutrient fluid, and pressure of the tissues, surrounding it. It is noticeable that the larger sarcocysts of a lamb of given age killed during the winter are in the looser, more vascular portions of the heart muscle. Likewise the smallest sarcocysts of such a lamb are found in the more compact and denser regions of the heart tissue. Consequently to draw conclusions from the study of a few sarcocysts will not be satisfactory, and up to this time there has been no opportunity to study a large body of controlled material taken in the spring or summer. Still, some of the results are interesting.

An old ewe, No. 226, was killed June 13. A very careful examination showed that the smallest sarcocyst found had a volume of 102,513 cubic micra. Evidently this parasite belonged to infection during a preceeding season, for one would expect to find much smaller sarcocysts if infection had begun the current spring. Again, the smallest sarcocysts found in March (Group 5, Table 3) had volumes between 53,000 and 60,000 cubic micra, and one would expect these to have a volume in June of over 100,000 if they increased in size at an average rate (see Table 3 for average rates of growth). So if one allows six weeks for the infection to appear in the muscles, reinfection had not begun (Ewe 226) on May 1. However, one must not overestimate this evidence for only twelve sarcocysts were found in the material preserved from this ewe; she had had small chance of infection recently and she had not been outside of a dry feed lot for more than twenty months. Perhaps if this ewe had been running on the range some small sarcocysts would have been found.

Two lambs, 986 and 996, one born March 26, and the other April 3, were killed June 22. No sarcocysts were found in either of these lambs. For a considerable portion of the latter part of April and during May they had access to pasture conditions. Beginning the first week in June they were kept in a dry lot until killed. If infection occurs without the necessity of any other host, one would expect to find sarcocysts in these lambs, since they were both old enough to show the infection and had been exposed to pasture conditions. Inci-

dentally, it may be mentioned that, owing to a late season, practically no insects were present up to the date when they were killed. Lamb 302, born early in June, died July 20. On June 13 this lamb had been taken from the ewe to be raised by hand, and had no contact with sheep after this date. It did not begin to eat grass to any extent until about the end of June, and if it became infected, the sarcocysts had not yet appeared in the muscles. Lamb 286, born about the middle of May, was kept in a dry lot until it died, July 28. No sarcocysts were found. It had been fed certain insects on July 17, 20, 24 and 27. Conditions for infection were not favorable, and this negative result is of no value. Lambs 284 and 294 born in May were killed August 9. They were kept in a dry lot until July 16, after which they were turned out to graze in a pasture where 100 per cent. infection had always been obtained in all lambs. These lambs, had, therefore, probably become infected, but the sarcocysts had not yet appeared in the heart muscle twenty-four days after their first exposure to favorable conditions for infection. Lambs 283 and 291, born in May, were killed September 22. These lambs were treated like 284 and 294, but were allowed to run in the pasture forty-four days longer, a total of sixty-eight days in pasture. In the material sectioned, one sarcocyst with a diameter of 15.4 and a length of 44μ was found in the heart muscle of lamb 282. So far as studied, no sarcocysts were found in lamb 291. The size of the sarcocyst indicates that this infection could not have occurred much, if any, later than Agust 1, assuming it takes forty days for the parasite to become established in the muscle tissue. The evidence presented above with reference to how early in the spring infection begins, is of no great value. However, it appears that infection does not begin very early in the spring, and probably at least five or six weeks must intervene between actual infection and the appearance of the sarcocysts in the muscle tissue. Altogether, *it is very clear that there is a distinctly marked seasonal infection,* and this fact is in agreement with the idea suggested in a former paper that *Sarcocystis tenella* may be primarily dependent upon some invertebrate, probably some insect host. But the solution of the question whether Sarcosporidia are aberrant forms of Neosporidia as maintained by Darling (1915) and supported by Scott (1915), or are identical with Cnidosporidia, as suggested by the observations of Piana (1896) and Galli-Valeria (1916), must depend upon other data. It is believed that certain experiments now in progress will throw light on this problem.

Since infection is hard to control, and since practically all sheep of this region are infected, the theory has been suggested that infection was possibly transmitted *in utero.* There is strong evidence against this idea. A lamb, No. 301, died shortly after birth. The mother was killed soon afterward and proved to be an old heavily infected ewe. No sarcocysts were found in the lamb. In our experiments other older

lambs, from infected mothers, have also been found free from infection. In this connection considerable work has been done. Bertram (1892) examined the embryos of sheep, swine and cattle, but found no sarcocysts in any of them.

Bergmann (1913) found sarcocysts in 8 per cent. of lambs 6 to 10 weeks old, but he found that 20 per cent. of lambs just a little older (3 months) were infected. M'Gowan (1914), who believes in congenital infection, examined in 1913 "a large number of embryos from heavily infected mothers," but nevertheless found no infection; he also examined a number of lambs at various ages and the earliest age at which he found infection was 3 months. In the following year he made a more critical examination, by means of serial sections, of lambs 2, 16, 18, 22, 27 and 29 days old, but found no sarcocysts. All the evidence is against the theory that infection occurs *in utero*. The gestation period of the sheep is nearly five months, and considering the cotyledonous type of placenta at least ten or twelve weeks of the period should be favorable for infection. If six weeks is added to the period favorable for infection, since no lambs under 6 weeks have been found infected, sixteen or eighteen weeks appears as probably the shortest time from infection until the appearance of the parasites in the muscles. But in the case of *S. muris,* according to Smith and Nègre, the time from infection to appearance of the parasites in the muscles may be as short as forty-five days, and there is no apparent reason why the time for *S. tenella* should be longer. Now Bergmann is the only investigator who has found sarcocysts in the muscles of lambs at an age approximating this short period, namely in lambs 6 to 10 weeks old. It then appears that infection after birth agrees with all facts yet brought forward, and all the evidence is opposed to the idea of infection before birth.

One may now consider the question, does infection occur only in young lambs, or does it recur in successive seasons? The proof is apparently conclusive that the latter alternative is correct. In the old ewes one finds small sarcocysts, and in addition large ones that are immensely larger than any of those found in lambs during the first year. This proves that infection extends over more than one year; that is, infection may and probably does recur in successive seasons as long as the sheep lives. An inspection of Table 4 will help to make this matter clear.

In the case of Ewe 226, killed about the middle of June, the volumes of the nine parasites given apparently fall in three or four groups, and so probably represent infection in three or four different seasons or years. Parasites 1 to 4 no doubt belong to infection one year back; parasites 6 to 8 are probably in their second or possibly third year; and parasite 9 is separated widely from number 8, and is

seemingly of older growth, perhaps about 3 or 4 years old. Since the largest sarcocysts found in lambs killed in March (Group 6, Table 3) were less than 600,000 cubic micra, sarcocyst 5 may be only about one year old, but it probably belongs to the second season preceding. However, one cannot determine with certainty the age by the size of

TABLE 4.—TO ILLUSTRATE THE VOLUMES OF SARCOCYSTS IN OLD EWES AND TO SHOW THAT INFECTION EXTENDS THRU MORE HAN ONE SEASON

Sarcocyst Number	Ewe No. 226 Killed June 13	Ewe No. 4 Killed October 24	Ewe No. 717; Killed December 28		
	Volume	Volume	Volume	Sarcocyst Number	Volume
1	102,513	4,702	27,684	51	298,144
2	182,023	5,712	30,178	52	299,625
3	185,526	6,701	38,304	53	309,217
4	185,856	6,751	42,592	54	327,703
5	644,335	9,820	45,999	55	332,750
6	1,018,484	18,817	57,584	56	332,750
7	1,354,896	56,453	61,327	57	340,139
8	1,741,610	90,626	64,420	58	383,029
9	2,861,932	93,828	71,874	59	404,624
10	.....	96,341	89,975	60	412,280
11	.....	126,737	92,248	61	417.404
12	.....	133,816	119,700	62	439.357
13	.....	134,853	119,700	63	446,880
14	.....	136,294	119,700	64	450,410
15	.....	137,773	122,998	65	453,508
16	.....	146,232	122,998	66	484,183
17	.....	147,581	124,104	67	519,090
18	.....	156,119	125,390	68	545,177
19	.....	157,767	125,390	69	578,985
20	.....	169,458	130,842	70	622,908
21	.....	172,497	131,098	71	665,000
22	.....	180,495	131,769	72	704,365
23	.....	234,890	137,998	73	709,582
24	.....	249,356	143,748	74	776,354
25	.....	275,272	143,748	75	795,659
26	.....	283,947	144,472	76	851,840
27	.....	297,369	144,812	77	862,488
28	.....	311,364	146,143	78	863,755
29	.....	325,075	153,863	79	875,844
30	.....	338,875	172,327	80	1,042,720
31	.....	372,956	172,491	81	1,120,234
32	.....	377,013	172,497	82	1,225,017
33	.....	429,143	179,685	83	1,245,816
34	.....	453,955	179,902	84	1,333,312
35	.....	456,744	180,241	85	1,596,000
36	.....	543,250	183,144	86	1,697,376
37	.....	561,009	184,497	87	1,770,602
38	.....	732,674	189,747	88	1,788,864
39	.....	754,252	190,812	89	1,862,400
40	.....	765,215	193,197	90	1,931,160
41	.....	766,656	196,196	91	2,793,100
42	.....	766,656	204,441	92	2,863,436
43	.....	1,072,599	206,841	93	3,194,400
44	.....	1,384,825	212,960	94	3,194,400
45	.....	1,397,439	225,142	95	4,599,936
46	.....	1,893,959	245,326	96	5,398,536
47	.....	1,992,623	250,360	97	6,673,340
48	.....	.....	268,329	98	6,965,977
49	.....	.....	281,107	99	9,171,411
50	.....	.....	281,107	100	16,959,640

the parasite, after the first year, on account of the different rates of growth of different sarcocysts. At the end of the second year the largest parasites of that year are overlapping in size the smallest due to infection the first year. By accumulating a vast amount of data one could possibly determine the probable age of each sarcocyst. Ewe 226 was more than 3 years old, but her exact age is not known.

From range Ewe 4, killed October 24, there was obtained a larger series of sarcocysts. A comparison of the volumes of the forty-seven sarcocysts measured with the volumes of the sarcocysts of the experimental lambs shows that the parasites of this ewe probably owe their origin to infection during three different seasons. Sarcocysts 1 to 22, and possibly 23 and 24, belong to the summer just past; sarcocysts 25 to 42 are probably in their second year, while 44 to 47, and probably 43, are in their third year. In any event the measurements show that infection has taken place in more than one season. No information was obtainable in regard to the age of this ewe. In reference to this group of sarcocysts another fact is of importance. The smallest sarcocysts found (Table 4) are about the same size as the smallest sarcocysts found in the experimental lambs killed October 27 to November 17. That is, infection occurs about as late in the season in old ewes as it does in lambs.

A still larger series of parasites was measured from Ewe 717, which was killed on December 28 at the age of 3 years and 9 months. This ewe had, therefore, been exposed to four seasons of infection. By a comparison with the results obtained with the lambs killed at different dates, the average size of the parasites of the fourth, that is, the immediately preceding summer, should be about 132,000 cubic micra. By a comparison with the largest sarcocysts found in lambs killed January 13, parasites 1 to 45 (Table 4) all belong to this fourth season. By a similar comparison, parasites 46 to 79, inclusive, with volumes varying from 245,326 to 875,834 cubic micra, should probably be assigned to the third, or second, preceding season. Sarcocysts 80 to 94, with volumes varying from 1,042,720 to 3,194,400 cubic micra, would seem to belong to the second, or third, preceding summer, and parasites 95 to 100, inclusive, probably belong to the first, or fourth, preceding season of infection. Here too it is evident that infection extended through more than one season. A study of Table 4 brings out another point of interest. If ewe 717 was infected during four different seasons as it had a chance to be, then there could be four groups of sarcocysts with approximately the same number in each group. But if these observations are based upon adequate data and the reasoning has been correct, it appears that the older the group, the smaller the number of sarcocysts in that group. This has been found to be true in other cases besides the three ewes given, and may be accounted for in one of three ways. Either the sarcocysts gradually disappear by disintegration, as they grow older; break up into smaller sarcocysts; or some of the older sarcocysts never reach more than a moderate volume. I have no proof for or against the first alternative, there is some evidence against the second, and certain observations indicate the third view is correct. After examining many hundreds

of sarcocysts I cannot say that I have found any evidence that sarcocysts break up by disintegration. If older sarcocysts break up, thus setting free spores which wander out and become seats of new infection, this procedure in the light of facts brought out in this paper, must be an annual periodic occurrence. Aside from the lack of plausability, the proportion of small to larger sarcocysts is not compatible with such a theory. While occasionally a sarcocyst shows a stricture on one side or at one end, I have never found such a stricture approach complete separation, nor does one find sarcocysts in pairs or in fours as one would expect if such fragmentation or division of the sarcocyst did occur. Fantham and Porter, referring to their figure 55 which represents a dozen or more young pansporoblasts, state that "at this stage pansporoblasts (sometimes called sporonts) may wander out and start new infection." Even if this is true, all sarcocysts pass far beyond this stage before the second summer approaches, and another explanation will need to be given for seasonal infection. Finally, in support of the third view it has been shown that in lambs of known age the size of the sarcocyst bears a close relation to the nature of the tissue surrounding it. This is true for the first season of infection, and it would seem reasonable to believe it holds true for later stages of growth. In this way one can account for the apparent gradual decrease in the number of parasites in the older groups. Another fact supporting this view is that in some old ewes the number of sarcocysts seemingly derived from the last preceding season of infection appears rather large as compared with infection in lambs that have passed thru only one summer. In any event, it is evident that *ewes become infected year after year in successive seasons.*

TABLE 5.—TO SHOW DIMENSIONS OF SOME OF THE SMALLEST SARCOCYSTS MEASURED

Number of Lamb or Ewe	Date Killed	Diameter, Micra	Length, Micra
Range lamb 3	July 13	11.0	57.2
Range lamb 5	Aug. 24	13.2	33.0
Exp. lamb 283	Sept. 22	15.4	44.0
Range ewe 4	Oct. 24	6.6	107.9
Range ewe 4	Oct. 24	8.8	61.6
Range ewe 4	Oct. 24	9.3	78.0
Exp. lamb 280	Nov. 3	9.4	68.0
Exp. lamb 263	Nov. 10	11.0	44.0

Perhaps a mention should be made of the size of some of the smallest sarcocysts found. The dimensions given in Table 5 above will serve as a basis of comparison with the findings of other investigators. The smallest sarcocyst of *S. tenella* observed by Bertram was 6μ wide and 47μ long. This is smaller than any of those given in the table, but all of those given were apparently in the so-called Bertram stage. Crawley claims to have found a cyst consisting of a single

dividing sporoblast, and another which contained eight sporoblasts, both located intracellularly. This apparently proves that the elements in a sarcocyst undergo schizogonous multiplication. Fantham and Porter have seen all stages in this division which occurs by longitudinal fission, and they describe the growth and extension of a Sarcosporidian in a vertebrate host as follows: "Each spore contains an amoebula which finds it way into a muscle. The amoebula grows and its nucleus divides, thus becoming an elongate, multinucleate mass. Around each nucleus the protoplasm segregates, and a number of young pansporoblasts are formed. At this stage pansporoblasts (sometimes called sporonts) may wander out and start new infections (Fig. 55). Later, partitions or septa are formed between the pansporoblasts. Several spores are ultimately found in each chamber, having been formed from the pansporoblast." Judging from this description, and their figure 55, the stage at which migration may occur is the so-called Bertram's stage of various writers. The sarcocysts listed in Table 5 were all approximately in this stage, tho two or three showed the protoplasm had not yet become definitely segregated around the individual nuclei. From the data available from all sources it is probable that these parasites in Table 5 represent stages from six to nine weeks after infection.

DISCUSSION AND SUMMARY

Reference has already been made to the report of M'Gowan (1914) who wrote this lengthy paper with the purpose of showing that the disease "scrapie" is associated with and probably is caused by Sarcosporidiosis. Tho a careful reading of his paper leaves one unconvinced, this thesis leads him to postulate a partial theory in reference to the life history of *S. tenella.* As stated above, a part of this theory involves infection *in utero,* and I have presented evidence to show that this is certainly not the usual method of infection. Aside from the data which demonstrate recurring seasonal infection, the youngest age at which *S. tenella* has been found in lamb muscle is amply sufficient to account for infection after birth. No one has found sarcocysts present in lambs under 3 months old, except Bergmann, who found them in a small percentage of lambs that were somewhere between 6 and 10 weeks old. One does not know just how soon after infection *S. tenella* may appear in the muscles, but it is known (Smith, Nègre, Erdmann, Crawley) that *S. muris* appears in the muscles 40 to 50 days after infection, and there is no reason why *S. tenella* should require a longer period. Many facts are opposed to the idea of infection *in utero,* and not a single fact has been adduced incompatible with the theory that infection occurs after birth. In this connection it may be well to examine the experiment that M'Gowan gives as a crucial test.

In April and May (1913) "four lambs, from four scrapie sheep, were obtained *as soon as they were born,* before the mothers had even licked them. They were removed at first to a large byre where no sheep had ever been before, and later to a field where a similar condition prevailed. No sheep were ever allowed near them. They were looked after by an attendant whose duties did not bring him in contact with other sheep. They were brought up on cow's milk until they were old enough to live entirely on grass. When they were about one month old, living keds from scrapie sheep were applied to two of them, and these two were kept apart from the others. No further step was taken until January, 1914, in order that if sarcocysts did develop there would be no doubt of their actual presence. Then pieces of muscle were examined from the gluteal region of all four, *and in all four fully-developed sarcosporidial cysts were found in as large numbers as in lambs from scrapie mothers and of the same age brought up under natural conditions.*" . . . "and from the experiment it would appear that no conclusion could be drawn from it other than that the parasite is passed on by congenital infection of the lamb from its mother."

This conclusion might be justified if the experiment did not admit of other explanations. If Darling's insect theory of infection is correct, one would expect just as heavy infection in these lambs as in lambs which ran in fields with their mothers. Again, if infective spores are set free in the feces, as is true of *S. muris,* the possibility of infection in this manner is not entirely excluded. For, some one *brought the living keds from scrapie sheep,* and evidently here was a chance for infection by contamination. While this experiment shows that sarcosporidial infection takes place independent of the sheep tick, it affords no evidence that cannot be explained as well, or better, by theories of infection after birth.

Fantham and Porter are quoted above with reference to an early stage in the development of a sarcocyst at which the pansporoblasts may wander out and start new infections. Granted this is a common method of multiplcation, it will not serve to account for seasonal reinfection, for the sarcocysts have passed beyond the pansporoblast stage before the second season arrives. It is hardly probable, that this is the usual method of reinfection, since one would not expect such an internal method of multiplication to cease on the approach of winter. M'Gowan's theory of multiplication and reinfection is even less probable. According to this author, the chromatin granules in a spore escape by bursting of the spore, and play a part in transmission of the disease (scrapie) both endogenously and exogenously. Our knowledge of the Sarcosporidia indicates that the spore is the unit of

infection, and even if his theory were possible it would be hard to reconcile it with what is now known of the seasonal character of infection and reinfection. On the whole, considering in particular the work of Nègre, Erdmann and Crawley, it would appear that the source of infection is external and by the way of the alimentary canal. The experimental evidence brought out in this as well as in a preceding paper is in favor of the same conclusion. In fact, it is hard to see how seasonal infection could depend upon anything other than external conditions, either directly or indirectly, and since it is known that certain sarcosporidial spores enter the body by way of the alimentary canal, this is most likely the usual avenue of infection.

M'Gowan examined a large number of sheep and lambs, and incidentally produced some data that correlates with seasonal infection. Between January 21 and April 29, 1913, he found sarcocysts in 553 out of 818 sheep (67.6 per cent.), all of which were about one year of age or older. This shows that infection is the prevalent condition among sheep of that region. Between April 30 and June 11 he examined 121 February lambs and found sarcocysts in only four, or 3.3 per cent. Allowing forty to fifty days after infection for the sarcocysts to appear in the muscles, it is probable that this percentage would have been larger if there were no external conditions influencing the time of infection. At the same time the data agrees nicely with the idea of seasonal infection.

A discussion of some of the larger aspects of the relation of seasonal infection to the life history of *Sarcocystis tenella* will be deferred until a later paper. It seems entirely probable that infection occurs by way of the alimentary canal. It is clear that sheep at any age are susceptible to infection, and seasonal infection does not appear to be due to any condition within the sheep's body. Being so, do these conditions depend directly upon climatic factors, such as temperature? Or do they require an intermediate host present only at certain times of the year? The answer to these questions will depend much upon the answer to what is the life history of the parasite outside of the muscles of the host, a question which is yet unanswered. Successive seasonal infection is fatal to the theory of infection *in utero*. If there is a stage, or phase of *S. tenella* in the intestine which results in the freeing of spores, as Nègre (1907) has shown to be true of *S. muris*, it would seem that temperature may be a direct prominent factor in controlling seasonal infection. And yet since the encysted spores remained alive for thirty days in dried feces and resisted a considerable degree of heat, it does not seem possible on this theory to explain the entire absence of winter infection in *S. tenella* unless the spores are extremely sensitive to cold, which is improbable. There is no other

known climatic factor in the region of the Laramie Plains that could bear a direct causal relation to seasonal infection. Equally improbable on the same grounds is the theory that a carnivorous intermediate host is necessary. Other facts opposed to the latter view were presented in a former paper.

It would seem then that seasonal infection bears only an indirect relation to climate. If so, the factors that determine infection in turn must depend upon climate. To fulfil these conditions an intermediate or more likely, a definitive host is required. Crawley (1916) believes the Sarcosporidia should be classed under Telosporidia rather than as Neosporidia, basing his conclusion on what is now known of the life history of *S. muris* and certain young stages that he found of *S. tenella.* More needs to be known of the life history of these forms before this question can be settled definitely. Considering the widespread occurrence of Sarcosporidia in herbivorous animals, such as the sheep, Crawley also believes a second host is obligatory. This is probably correct, but his hypothesis with reference to a carnivorous animal must be rejected. Under the conditions of our experiment it is not possible to account for such infection as I have obtained on the theory of a carnivorous intermediate host, whether it be dog, cat, rat, mouse or ground-squirrel. If then a second host is necessary for *S. tenella,* the best remaining hypothesis is to look for this host among the invertebrates and a former paper has given reasons for believing this host would be found among the insects. The seasonal dependence of insects is also in accord with the facts presented in this paper.

There is a second hypothesis that must be taken into consideration. Nègre has shown that the feces of a mouse infected with *S. muris,* become and remain infective for a long time, from the fifteenth to the sixtieth day. Crawley has verified this work, and while the bodies that cause the infection have not been actually observed, they are known to exist in the feces. If there is a similar stage in the life-history of *S. tenella* the fragile character of the spores, which has been noted by Fantham and Porter, may be sufficient to account for seasonal infection. The dry, cool climate of this region, frequently becoming quite cold in the winter may soon kill the spores during a portion of the year. However, there are many mild periods in winter which should not be very destructive, and the lambs of the experiments noted in this paper ran, fed and watered with their ewes, so they had abundant opportunity to become infected by contamination. Yet there was not a single case of winter infection. Either the spores are set free at only certain seasons, or there is some doubt in regard to this second hypothesis. Experiments are now in progress which will probably show which of the two hypotheses is more nearly correct.

SUMMARY

The chief points of this paper may be summarized as follows:

1. There is a well-defined seasonal infection of *Sarcocystis tenella* in the region of the Laramie Plains. It is not known whether this is true or not of other regions. Young stages of this parasite have been found in the muscles of both sheep and lambs thruout summer and early autumn, but not during the winter and spring.

2. Reinfection occurs in successive seasons, and old sheep are apparently as susceptible to infection as are young lambs. The theory of infection *in utero* is untenable. Seasonal, self-reinfection is improbable, tho not entirely excluded, and the evidence indicates the origin *de novo* of successive infections.

3. If a second host is required, which seems probable, it is very likely that this host is an insect, and that the definitive (sexual) stage of the parasite will be found here.

4. If a second host is not necessary, the sexual stage probably takes place in the intestine of the sheep, and in some unknown way the life cycle falls under the influence of seasonal control.

5. In old ewes the larger sarcocysts are not nearly so abundant as the smaller ones. That some of the older sarcocysts do not grow to a large size is probably the most satisfactory explanation of this fact.

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THE EFFECT OF LAUNDERING UPON LICE (*PEDICULUS CORPORIS*) AND THEIR EGGS*

WILLIAM MOORE

Division of Entomology and Economic Zoology, Department of Agriculture, University Farm, Saint Paul, Minnesota

At the request of Dr. Richard M. Pearce of the National Research Council, on July 25, the author took up the question of the effect of the ordinary steam laundry processes upon lice and their eggs. The object of the investigation was to determine to what extent these processes were destructive to both lice and eggs, and should they prove to be inefficient, what slight alterations could be made in the regular routine to make them effective.

LAUNDRY PROCESSES

Through the courtesy of Mr. J. Clair Stone, manager of the Elk Laundry, St. Paul, I was able to study the processes encountered in the washing of regulation army clothing. The clothing may be divided into three types: rough cotton goods (including cotton underwear), cotton khaki wear, and woolen goods (including garments part wool and part cotton).[1] Altho the procedure differs somewhat in different steam laundries, they may in general be outlined as follows:

	Baths	Temperature	Time
Cotton Goods	1st Water	100° F. (37.7° C.)	5 min.
	2nd Neutral Soap	180° F. (82.2° C.)	15 min.
	3rd Neutral Soap	180° F. (82.2° C.)	15 min.
	4th Soda Bath	130° F. (54.4° C.)	10 min.
	5th Water	130° F. (54.4° C.)	5 min.

Cotton goods are dried in the hot air tumbler at a temperature of 150° F. (65.5° C.) to 190° F. (87.7° C.) until quite dry. Time about 20 minutes depending upon the load.

	Baths	Temperature	Time
Cotton Khaki	1st Water	100° F. (37.7° C.)	5 min.
	2nd Neutral Soap	120° F.-130° F. (48.8° C.-54.4° C.)	15-20 min.
	3rd Water	130° F. (54.4° C.)	5 min.

Dried in the hot air tumbler at 150° F. (65.5° C.) to 180° F. (82.2° C.) until just sufficient moisture is left in the garment that it may be pressed. Time about 10 to 15 minutes depending upon the size of the load. Pressed in the Universal Press.

	Baths	Temperature	Time
Woolen Goods	1st Neutral Soap	110° F.-115° F. (43.3° C.-46.1° C.)	15 min.
	2nd Water	110° F.-115° F. (43.3° C.-46.1° C.)	3 min.

Woolens are dried at room temperature and never in the hot air tumbler.

[1] Work done at the suggestion and with the support of the Medical Division of the National Research Council.

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The first important point to determine was what effect the temperatures encountered would have upon the lice and nits. Data were available from the work of other investigations giving an indication of what results might be expected. The following table was taken from a compilation of Nuttall (1918).

IMMERSION OF EGGS IN HOT WATER

Temp., Degrees F.	C.	Time	Result	Observer
192	88	15 sec.	Killed	Nuttall
169	76	30 sec.	Killed	Nuttall
158	70	10 sec.	Killed	Nuttall
150	67	1 min.	Killed	Nuttall
140	60	5 min.	Killed	Nuttall
140	60	5 min.	Killed	Widmann
131	55	10 min.	Killed	Widmann
131	55	30 min.	Killed	Bacot
129	54	10 min.	Killed	Nuttall
121	50	15 min.	Killed	Widmann
112	45	15 min.	Not Killed	Widmann
104	40	1 day	Not Killed	Widmann

EXPOSURE OF EGGS TO DRY HEAT

124	51.5	15 min.	Not Killed	Experiments of Capt. Orr, Canadian A. M. C., and Bacot
127	53	15 min.	Not Killed	
130.5	55	30 min.	Killed	
132.5	56	20 min.	Killed	
134	57	30 min.	Killed	
152	57	15 min.	Killed	

EXPERIMENTS

In my experiments, it was found that the quantity of soap used varied somewhat due to the hardness of the water. Sufficient soap was added to the water to give a good suds. It was found that with the water used in the experiments recorded below that 1 gram of ivory soap (neutral) and ⅓ gram of soda added to 265 c.c. of water furnished the desired suds. Inasmuch as the eggs are more difficult to destroy than the active stages, particular attention was paid to them. All the eggs were from lice collected from infected clothing, and kept in an incubator heated to 28° to 32° C. The eggs were laid upon small squares of cloth during the week of July 27 to August 2, in Exp. 1 to 6, and from July 27 to August 7 in Exp. 7 to 12. Each piece of cloth therefore represented eggs in different degrees of development.

Experiment 1.—Control set: 42 eggs; 78½% hatched.

Experiment 2.—Woolen Goods Treatment. Soaked in suds heated to 110° F.-114° F. (43.3 C.-45 C.) for 15 minutes. Rinsed in water of same temperature for 3 minutes, dried on a piece of filter paper and returned to the incubator; 65 eggs 92% hatched.

Experiment 3.—Khaki Wear Treatment. Soaked in suds heated to 121-126 F. (49-52.2 C.); average temperature, 123 F. (50.5 C.), for 15 minutes. Rinsed in water 123 F. (50.5 C.) for 4 minutes. Dried and returned to incubator; 38 eggs 39% hatched.

Experiment 4.—Khaki Wear Treatment. Same as Experiment 3 except treatment was for 30 minutes. 45 eggs, 0% hatched.

Experiment 5.—Cotton Goods Treatment.—Soaked in suds at 170-186 F. (76.6-85.5 C.), average temperature 179 F. (81.6 C.), for 30 minutes. Rinsed in water 130 F. (54.4 C.) for 5 minutes. Dried and returned to incubator; 52 eggs, 0% hatched.

The following experiments were conducted to determine the effect of treatment in the hot air tumbler and pressing in the Universal Press upon the eggs of the louse.

Experiment 6.—Eggs placed in pocket of a bathrobe in the hot air tumbler carrying a heavy load. Tumbler had been running for 5 minutes before eggs were placed in it. Eggs in the tumbler for 10 minutes and garments were quite moist when eggs were removed. Eggs replaced in incubator after treatment. 88 eggs, 0% hatched.

Experiment 7.—Control, 48 eggs. 100% hatched.

Experiment 8.—Cloth, upon which the eggs were laid, wet and then placed in the pocket of a pair of khaki trousers which was tumbled with others for 15 minutes. Load light and removed while still damp. Regular practice of drying khaki wear. 146 eggs, 0% hatched.

Experiment 9.—Eggs placed in pocket of partly dried bathrobe. Light load of clothing, tumbled for 10 minutes. 53 eggs, 0% hatched.

Experiment 10.—Same as Experiment 9, but tumbled for 15 minutes; 73 eggs, 0% hatched.

Experiment 11.—Same as Experiment 10, but tumbled for 20 minutes; clothing quite dry when removed. Regular cotton goods treatment. 57 eggs, 0% hatched.

Experiment 12.—Cloth with eggs placed under pocket of a pair of khaki trousers being pressed in the Universal Press. After treatment removed to incubator. 61 eggs, 0% hatched.

The recorded experiments upon the effect of soap suds at different temperatures upon the eggs of the lice would lead one to suppose that active stages would also be destroyed in those experiments where the suds had proved destructive to the eggs. To verify this, the following experiments were conducted.

Experiment 13.—Twelve recently fed lice in different stages of development were dipped in suds at 110-114 F. (43.3-45 C.) for 15 minutes, rinsed in water at 112 F. (44.4 C.) and dried on filter paper. All revived within a few hours.

Experiment 14.—Same as Experiment 13, but suds at 122-126 F. (50-52.2 C.), average temperature 124 F. (51.1 C.) for 15 minutes. All lice killed by treatment turning reddish brown within 5 hours.

Experiment 15.—Same as Experiment 14, but exposure lasting 30 minutes. All lice killed.

The experiments show that in the washing of rough cotton goods at 180° F.—82.2° C. for 15 or 30 minutes, will destroy the lice and their eggs. If by any chance an egg should escape destruction in the washing process they would later be destroyed during drying in the hot air tumbler. Washing cotton khaki clothing at a temperature of

120° to 130° F. (48.8° to 54.4° C.) for 15 minutes would prove destructive to the active stages, but would not completely destroy the eggs. Washing for 30 minutes, however, proved destructive to the eggs. Drying khaki uniforms in the hot air tumbler would also destroy any eggs that might have escaped the action of the hot suds. Pressing in the Universal Press was also effective, but this treatment cannot be relied upon to destroy all the eggs in an infested suit as portions of the uniform may not be touched. Neither the lice nor their eggs were destroyed in the woolen goods by the regular washing and since they are dried at room temperature, to avoid shrinkage, the problem resolved itself into devising some method of laundering woolens that would prove destructive. The first method which suggested itself was the treatment of the woolen goods in the hot air tumbler for 10 to 15 minutes before they are washed and while still dry. Nuttall (1918) claims "that the moderate degree of dry heat necessary to kill vermin will not prove injurious to wool, but that high temperatures 104° C. acting for 4 hours whilst but slightly yellowing white flannel does not affect its tensile strength, but if exposed to 127° C. for half an hour, flannel yellows and becomes brittle." This method, however, is open to two objections; namely, the danger of reinfestation of clean garments from handling garments infested with active stages in the vicinity of the tumbler, and the coagulating effect of the hot air on stains of blood, excreta, and other proteins, which may be present on garments before they are washed. Both these objections would be removed if the garments were first washed in such a manner as to destroy the active stages. The garments after drying could then be run in the tumbler to destroy all eggs which had escaped destruction during the washing.

In other experiments on contact insecticides (Moore and Graham, 1918) it had been found that where the insecticide possessed both wetting and spreading properties, the insecticide entered the tracheae of the insect, thus bringing about its death. Fat solvents, oils, etc., together with soap possessed such properties. Ivory soap, however, was found to possess great cohesion, thus preventing it from readily entering the tracheae. By raising the temperature of the solution or diluting it with water, the cohesion was reduced. From these results, it was not apparent why the suds used in the previous experiments (13) at a temperature of 110° F. to 114° F. (33.3° to 45° C.) should not have killed the active stages of the lice. The following experiments were conducted to throw more light on this point.

Experiment 16.—Lice not fed for 5 hours were dipped in a ivory soap solution of 1 gram to 100 c.c. of water colored blue with trypan blue. Temperature 108-115 F. (42.2-46.1 C.). Lice removed in 15 minutes and examined by mounting in alcohol on a glass slide, but no trace of the colored soap solution could be found in the tracheae.

Experiment 17.—Same as Experiment 16, but soap solution 1:250 c.c., results negative.

Experiment 18.—Same as Experiment 16, but soap solution 1:500 c.c., results negative.

Experiment 19.—Same as Experiment 16, but soap solution 1:750 c.c., results negative.

Experiment 20.—Same as Experiment 16, but soap solution 1:1,000 c.c., results negative.

Experiment 21.—Same as Experiment 18, but soap solution at a temperature of 122-132 F. (50-55.5 C.). Lice were killed by the treatment but no trace of the solution could be found in the tracheae.

Experiment 22.—Lice placed in soap solution 1:500 at room temperature at 8:13 a. m. and removed at 3:30 p. m. No trace of soap solution in tracheae of specimens examined. Lice divided into two lots; one rinsed in water; the other not rinsed. Both sets revived within an hour.

Since it appeared impossible for the ivory soap solution to enter the tracheae, a solution of castile soap with much lower cohesion was used but similar negative results were obtained. Soap solutions having failed to enter the tracheae, the question arose as to whether fat solvents or oils could penetrate.

Experiment 23.—Lice dipped in xylene stained with sudan III were examined at the end of 5 minutes but no trace of the stain could be found in the tracheae.

Experiment 24.—Lice dipped in ether stained with sudan III. One specimen examined after two minutes but no stain was detected. Stained ether was found in few tracheae of a louse in the ether for 5 minutes but none was found in a specimen removed after 8 minutes.

Experiment 25.—Twelve lice dipped in ether stained with sudan III for 10 minutes. Examination showed 7 with no ether in the tracheae and 5 which had ether in several tracheae but none with ether in all the tracheae.

Experiment 26.—Four lice dipped in a light lubricating oil stained with sudan III. Removed after 15 minutes, but no stain could be detected in the tracheae.

Most of the lice in these experiments were dead when removed from the liquid, having been killed by the chemical passing directly thru the body wall, since no stain could be detected in the alimentary canal. Landois (1865) has figured the closing apparatus of the pubic louse which is similar to that of the clothes louse and from the above experiments, the conclusion is reached that the louse is able to close this apparatus very quickly, but occasionally, as in the case of ether, a few tracheae are not closed quickly enough to keep out the chemical. A few experiments showed that the tracheae of the dog flea (*Pulex serraticeps*) was filled with stained ether after 1 minute immersion, but that the hog louse (*Haematopinus suis*) and the dog louse (*Haematopinus piliferus*) were somewhat resistant to its penetration, but not nearly so successful as the clothes louse. It is hoped to investigate this interesting observation more fully at some later date.

Two possible methods of killing the active stages is suggested by these results: First, the addition of a chemical to the washing suds capable of penetrating the chitin of the body wall during the period of washing, and toxic enough to produce its death, and second, the elevation of the temperature of the washing suds sufficiently high to destroy the lice. In general, a chemical capable of penetrating the body wall during the period of washing would have to be rather volatile and hence not suitable for the work. Judging from published accounts, soaking garments in a bath containing cresol or lysol is practiced to a large extent in Europe. The garments, however, are not rinsed following their dip. Peacock (1916) found a 1½ per cent. cold cresol solution to be capable of destroying the lice and nits soaked in it for one hour. Nuttall (1918) found a 5 per cent. cresol and soap solution to kill lice and nits in 30 minutes, while a 2 per cent. lysol solution at 76° F. (24.3° C.) killed the eggs after 5 minutes exposure. Bacot and Lloyd (1918) considers that "the evidence as a whole seems to establish the fact that steeping for twenty minutes in a 2 per cent. solution, either lysol or the cresol soap, is quite effective provided the temperature is not below 50° F." The following experiments were conducted to determine the efficacy of cresol either as a dip preceding washing, or when used in the wash suds.

Experiment 27.—Dipped 12 recently fed lice in suds with 1% tricresol added. Temperature 75 F. (24 C.). Removed after 5 minutes to suds at 110-114 F. (43.3-45 C.) for 15 minutes, rinsing in water at 112 F. (44.4 C.) for 3 minutes. Dried on filter paper when 10 lice revived.

Experiment 28.—Same as Experiment 27, but cresol suds at temperature of 110-114 F. (43.3-45 C.), 9 lice revived out of 16 used in the experiment.

Experiment 29.—Dipped recently fed lice in 1% tricresol in ivory soap suds at 110-114 F. (43.3-45 C.) for 15 minutes, rinsing in water at 112 F. (44.4 C.) for 3 minutes. Dried when one revived out of 17 lice.

Experiment 30.—Dipped in 2% tricresol in suds at 110-114 F. (43.3-45 C.) for 5 minutes. Placed in regular suds at 110-114 F. (43.3-45 C.) for 15 minutes, rinsing in water at 112 F (44.4 C.). Dried, no lice revived.

Experiment 31.—Same as Experiment 30, but with 3% tricresol. All lice killed by the treatment.

From these results, it is apparent that 2 per cent. crude tricresol may be added to the washing suds or used as a dip preceding washing and prove effective in the destruction of the lice in the active stages. Although the pieces of cloth were rinsed after treatment, an odor of cresol persisted, apparently being rather difficult to remove.

Bacot and Lloyd (1918) point out that cresol emulsions are liable to decrease in insecticidal value in the presence of organic impurities. To what extent this action takes place is not known and possibly varies greatly. Such being the case and in view of the increased cost of

using a chemical to destroy the lice, further experiments were made to determine to what extent heat might be used. A summary of these experiments follows.

Summary of 30 Minute Treatments

Temperatures	Dead	Revived
108-110 F., Average 108.8 F., 42.6 C.....	1	7
110-113 F., Average 110.7 F., 43.7 C.....	3	7
110-115 F., Average 111.6 F., 44.2 C.....	1	16
109-115 F., Average 112.4 F., 44.6 C.....	15	1
110-115 F., Average 113 F., 45 C.....	18	0
112-114 F., Average 113 F., 45 C.....	15	0

Summary of 22 Minute Treatments

110-116 F., Average 112.8 F., 44.8 C.....	10	0
113-115 F., Average 114.2 F., 45.6 C.....	10	0
114-117 F., Average 115.2 F., 46.2 C.....	8	0

Summary of 15 Minute Treatments

111-115 F., Average 112.3 F., 44.6 C.....	6	8
111-115 F., Average 113 F., 45 C.....	11	0
111-115 F., Average 113.3 F., 45 C.....	9	9
112-116 F., Average 114 F., 45.5 C.....	10	2
112.5-116 F., Average 114.2 F., 45.6 C....	18	0
113.5-117 F., Average 114.9 F., 46 C.....	8	0
115.5-117.5 F., Average 116.5 F., 46.9 C..	6	0

These experiments show the lethal temperature for lice is about 113° F. (45° C.) for 22 to 30 minute washings, and a slightly higher temperature 114.5° F. (45.8° C.) proved effective in 15 minutes' time. When woolen garments are quite soiled, the usual practice in laundries is to wash them at the higher temperature of 120° to 125° F. (48.8° to 51.6° C.), care being taken to keep the temperature constant thruout the process which is the important point in washing woolens to prevent shrinkage. These temperatures may be easily maintained in the washing machine.

Considering the data presented, the following procedure is recommended for the laundering of woolen goods to destroy both lice and eggs. Infested garments to be washed at a temperature of 120° F. (48.8° C.) not to fall below 115° F. (46.1° C.) during the washing period of 15 minutes, this treatment to destroy the active stages without the use of any special chemicals. Garments are then treated in the regular manner until perfectly dry, when they should be placed in the hot air tumbler at a temperature of 150° to 170° F. (65.5° C. to 76.6° C.) for 10 to 15 minutes resulting in the destruction of the eggs. By this method, it will be possible to launder woolens without shrinkage, and destroy the lice and eggs without the use of a special chemical.

These experiments have been corroborated in general by the experiments conducted with the regular army laundering units by W. Dwight Pierce and Lieut. A. Moscowitz. In their experiments, the woolens were washed at a slightly higher temperature, 131° F., and dried in the hot air tumbler without shrinkage resulting.

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THE ANATOMY OF *TETRACOTYLE ITURBEI* FAUST, WITH A SYNOPSIS OF DESCRIBED TETRACOTYLIFORM LARVAE*

ERNEST CARROLL FAUST

Thru the courtesy of Professor Henry B. Ward, the writer has been enabled to examine specimens of *Planorbis guadelupensis* Sowerby infected with a new tetracotyle, to which the name *Tetracotyle iturbei* has been given (Faust 1918a). The material, for which I am greatly indebted to the kindness of Dr. Juan Iturbe, of Caracas, Venezuela, was at first considered to be "rediae" of *Schistosoma mansoni* (Iturbe and Gonzalez 1917). The infection occurs as cysts in the testicular cavities of the mullusk. As a result of the infection, these lumina are highly inflated, measuring two to three times normal size.

The material was examined by teasing out some of the worms and mounting as totos and by sectioning others *in situ*.

Description of *Tetracotyle iturbei* Faust 1918

Tetracotyle iturbei is a pear-shaped fluke measuring 0.42 mm. long, 0.33 mm. wide, and 0.3 mm. thick in the region of the primitive genital pore (Fig. 1). The oral sucker has a diameter of 52μ; the primitive genital pore, 42μ, and the acetabulum 95μ. Posterior to the middle of the oral sucker and lateral in position are the accessory suctorial grooves with their oval openings directed anteromesad. These organs are undoubtedly muscular and are deeply sunken in the tissue of the worm. The body as a whole is enclosed in a thin mucoid cyst capsule, fitting tightly around the tetracotyle everywhere except in the region of the ventral attachment organs. There is no armament anywhere on the integument. No inclusive suctorial cup, such as is described for *Cercaria flabelliformis* (Faust 1918), is found to surround the ventral attachment organs. In sagittal section the outline of the worm resembles a similar section of a trochophore larva.

From the deeply sunken oral sucker the alimentary tract leads dorsad. Immediately above the oral sucker is the flask-shaped pharynx, 16μ in trans-section. The ceca arise at the dorsal end of the pharynx and proceed posteriad after looping somewhat ventrad and then dorsad again to the plane which the posterior end of the pharynx occupies.

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* Contributions from the Zoological Laboratory of the University of Illinois, No. 125.

The ceca describe a wide furculum. They end blindly in the region of the anterior ventral sucker, or at times extend to the margin of the posterior ventral sucker. These diverticula are composed of a single layer of granular cells surrounding a small lumen. They have no suggestion of muscular elements. The cells of the ceca are cuboidal, about 4 to 5μ thick, with spherical nuclei 2μ in diameter, located in the center of the cells.

Only the main trunks of the excretory system were made out, and those with great difficulty. The material which was available for study was preserved in formol, so that none of the ultimate traces of the system were left. The bladder is situated dorsal to the genital pouch; it is nonmuscular and inconspicuous. Emptying into it are two swollen trunks which occupy the greater part of the body lateral to the digestive ceca. As far as they can be made out they have no cellular lining, but are merely lumina within the parenchymatous complex of the worm. These trunks extend as far anteriad as the lateral suctorial grooves. They probably branch here, but the details cannot be followed. No excretory granules, such as are usually found in the Holostomidae, have been preserved in this specimen.

The nervous system of the holostomes has been remarkably altered, synchronously with the evolution of this group in other directions. The working out of this system was so difficult that Brandes (1891: 570) passed over its consideration in a brief paragraph, merely stating that he had observed nerve elements in the parenchyma of the suckers.

The present species has a nervous system similar in all essentials to that of *Cercaria ptychocheilus* (Faust 1918: 54, 55), but shows some interesting minor differences. In *Tetracotyle iturbei* the brain mass is large and is situated some distance dorsal to the pharynx (Fig. 4). There is a thick dorsal commissure. Anterior to it are dorsal and ventral trunks and perhaps traces of intermediate lateral trunks. From the posterior ventral angles of the brain are derived two paired trunks, dorsales and ventrales; and, in addition, a subesophageal commissure. The posterior dorsal trunk is fairly compact, cylindrical, and measures about 6μ in cross section. On the other hand, the posterior ventral trunk is very diffuse, being much larger than the dorsal ramus, in all some 9 or 10μ in cross section. The individual nerve fibers in this trunk can be easily distinguished. Given off from the ventral margin of the subesophageal commissure is a median fused trunk. In the plane of the lateral suckers it forks so that the branches of each fork surround the muscular region below the primitive genital pore. The muscular organ is the primitive vagina and the nerve is the genital nerve. In the region of the primitive genital pore the posterior dorsal trunks lie lateral and ventral to the ceca, while the large unsheathed ventrales lie in the plane of the pore, just lateral to it.

Branches of these two nerves form a dense mat between the parenchyma and muscle elements, ending in the body wall especially in the region of the acetabulum and lateral suckers. Prominent branches of the ventrales continue posteriad to the posterior genital pouch. The sensory nerve fibrillae (Fig. 3) pass in between the longitudinal and transverse nerve fibers and end in small papillae in the inner granular region of the integument.

The nerve cells are confined to the brain mass. No cell walls can be made out, but the nuclei are readily seen. They are oblong-ovate or reniform, and measure 1.5μ in short diameter and 4μ in long diameter.

The genital organs are differentiated early in holostomes. Midway between the acetabulum and the posterior genital pore is the ovarian cell mass, 25μ in transverse diameter. Dorsally, it opens thru the short oviduct into the ootype (Figs. 1, 5). The vitelline glands are long cylindrical cords, reaching cephalad as far as the primitive genital pore, and describing a broad H, with the anterior arms much the longer. At their anterior end the cords lie just ventral to the posterior limits of the ceca. Short transverse ducts connect them with the ootype. The vitellaria are composed of large granular cells with vesicular spherical nuclei. Posteriad, the ootype opens into a narrow cylindrical tube which continues caudad and opens to the exterior somewhat ventral to the excretory vesicle. There is no distinct enlargement into a genital pouch as has been described for various adult species and for the larvae, *Cercaria flabelliformis* and *C. ptychocheilus* (Faust 1918: 110-112). Nor is there a definite muscular wall here. There are, however, numerous muscular elements which have their insertion posterior to the ootype and are spread out in fan-shaped arrangement, ending in the posterior body wall, muscles which undoubtedly serve in the capacity of dilating the genital atrium.

Pyriform testes, 50μ in trans-section, are found lateral to the ovary and slightly anterior to it. Their efferent ducts run caudad separately and open into the genital atrium from the sides. The testicular elements consist of polygonal cells with many chromioles and no well defined nuclei.

The anterior of the two ventral suckers is of the highest significance in the phylogenetic history of the holosomes. In the literature this attachment organ has been referred to as the acetabulum by Moulinié (1856) and later workers. The posterior ventral sucker has been regarded as an accessory suctorial organ, which most investigators have considered a creation *de novo,* but which Ssinitzin (1910: 19-21) has thought to be the modified genital pore. The latter investigator has seen a resemblance between the suctorial organ of *Holostomum erraticum* Duj. of Brandes (1891: Taf. 41, Fig. 5) and the

penial organ of his own unusual distome larva, *Cercaria plicata*. A most serious difficulty prevents such a conclusion, namely, that the genital organ of distomes is usually anterior or lateral to the acetabulum and in only a few species posterior to that organ.

Of all the writers on holostome anatomy and phylogeny, Odhner (1913) alone considers the posterior ventral sucker to be the acetabulum; the anterior ventral sucker he regards as a phylogenetically new organ. *Tetracotyle iturbei* provides ample evidence in support of the view previously proposed by the writer (1918) that the anterior ventral sucker is the primitive genital pore, while the posterior ventral sucker is the acetabulum.

A median sagittal section of the tetracotyle (Fig. 5) shows three openings on the ventral side, the oral and the two ventral suckers. The posterior ventral sucker is muscular. The outer part is funicular and leads into a large deep pocket which ends blindly ventral and caudal to the ovary. Likewise the anterior ventral sucker has a funicular opening. Within it there is a narrow tube, walled with a single thick layer of elongate cells leading dorsocaudad. Near the dorsal wall it opens into a U-shaped tube of large diameter lined with cuboidal cells of granular structure. This tube in turn opens into two genital organs, the ovary, caudoventrad, and the vagina, anteroventrad. The latter organ is large and irregular in contour, 37μ in thickness and 64μ long. It is walled with several muscular layers and has only a small lumen.

Hence *Tetracotyle iturbei* has two genital canals leading to the outside, one opening anterior to the ovary and just in front of the acetabulum, and the other opening caudad below the excretory pore. The genital canal opening thru the anterior ventral sucker proves this sucker to be a modified genital pore. On the basis of this direct evidence this sucker is to be regarded as the primitive genital pore of all tetracotyle and diplostomulum larvae, even where the connection with the genital organs has been lost. Furthermore, the undeveloped muscular elements of the posterior genital atrium in this species, together with the clear connection between the ootype and the primitive genital pore, suggest that this species is phylogenetically a transition form between distome and holostome types. The vagina is an organ not usually found in the holostome group. No Laurer's canal has been made out with certainty, but it probably arises from the dorsal wall of the glandular region along the primitive genital canal.

The study of the genital system in this species, then, contributes important evidence in support of the distome relationship of the holostomes. It shows the direct homology between the anterior ventral sucker of the holostome and the distome genital pore. In confirmation of Odhner's view it homologizes the posterior ventral sucker of holostomes with the distome acetabulum.

The encysted animal is covered with a thin but firm capsule of mucoid material of a bluish-gray hue. Beneath this is the integument. There is no epidermis present. Directly beneath the cyst capsule is a firm, almost homogeneous non-cellular layer, in which minute refractory granules are brought out by a very bright illumination. An equally thick layer of the same material lies just beneath this covering. It differs from the outer layer in being more diffuse and in having larger granules. The sensory nerve fibrillae penetrate into this layer and end in delicate papillae (Fig. 3).

In many regions of the body the parenchyma is almost obliterated by muscle and nerve elements. It may be stated with considerable certainty that little if any undifferentiated parenchyma remains in the larva at this stage of development. In the deeper regions of the body it has been converted into connective tissue. In the region next to the body wall the cells have long aciculate processes which penetrate thru the muscle layers into the inner integumentary layer. These cells probably function in the secretion of the integument.

The holostome larva as illustrated by *T. iturbei* is a unique example of muscular development. The muscles function primarily in the attachment of the worm to the tissues of the host and not in locomotion. The body wall has two series of muscles, an outer single layer of transverse fibers and many layers of longitudinal fibers just within the transverse layer. No muscles have been found in connection with the digestive ceca. The glandular elements of the acetabulum make it possible for this organ to function as a digestive organ. There is a strong pharyngeal sphincter around the esophagus, directly above the oral sucker.

The suctorial organs of this species all contain muscular elements. In most tetracotyles the acetabulum is described as possessing glandular elements. For the accessory lateral suckers of *Tetracotyle echinata* Diesing (1858: 367) and *T. petromyzontis* Brown (1899: 493, Fig. 5) definite granular structures have been described, but the glandular nature of these organs is probably of secondary origin and not their primary function.

Important retractor muscles are situated in two regions of the body. In the anterior part (Fig. 6), dorsal to the origin of the digestive ceca, a heavy double muscle band has its insertion. One part runs ventrad to the left of the pharynx and the other runs ventrad to the right of the pharynx. Each part of the band spreads out in fan-shaped arrangement so that it occupies the entire lateral region between the oral sucker and the genital pore. With the contraction of these muscles the entire region between these suckers is converted into a vacuum, by means of which the worm is intimately attached to the host. Muscle strips inserted in the region of the uterus of the worm have their

ending in the posterior wall. They probably function in the dilatation and contraction of the functional genital pore.

The muscle cell nuclei are usually spherical, with a diameter of about 4.5μ. In the region of the primitive genital pore, however, some are stellate. These nuclei are all abundantly filled with chromidia, which in some cases, are massed into karysomes.

Only encysted individuals of *Tetracotyle iturbei* have been found. Like the distomes, the holostome larvae have been shown to be heterogenetic (Faust 1918). It is expected, therefore, that the cercariae of this species are produced parthenogenetically within a redia or sporocyst.

DISCUSSION

Tetracotyle iturbei is the first larval holostome to be described from South America. Records for North America have been made by Leidy, Rettger and Faust. These records, as well as those for *Tetracotyle typica* Europe are from molluscan hosts. Other tetracotyles are recorded from leeches, fish, amphibians, reptiles, birds and mammals. In every case except that of *Cercaria flabelliformis* the larvae have been found in the encysted or postencysted state. The doubtful case of *Tetracotyle hirudium* (Schomburgk 1844) gives the single record of an ectoparasite in the group.

No end of confusion in the systematology of holostome larvae has resulted from a disregard for the original diagnosis of the genera together with ignorance of the life-history processes of the group. The genera Diplostomulum, Tyrodelphys, and Tetracotyle have been recognized, but species of each of these have been placed in each of the other genera by overlooking items in the original description and by substituting incorrect descriptions for the genera to fit the cases in hand.

In 1832 von Nordmann proposed the name Diplostomum for the flat holostome larvae with two ventral suckers and no accessory lateral sucking organs. He recognized two subgenera with the type species *Diplostomum volvens* and *D. clavatum*. Unfortunately, he failed to name the subgenera. Diesing (1850: 304) removed the *clavatum* type to a new genus, for which he proposed the name Tylodelphys.

Tetracotyle typica was described by Steenstrup (1845: 129; Taf. 5, Fig. 3) as a "true distomata, *Distoma tarda.*" The accessory suctorial organs were considered to be excretory organs. In 1855 de Filippi found the same species in conjunction with sporocysts of *Cercaria furcata,* and, recognizing the lateral organs as suckers, proposed the name Tetracotyle for the group. The name for the species described by Steenstrup and de Filippi, as proposed by Diesing, is *Tetracotyle typica* (1858: 366). In as far as this larval group can be recognized as a genus, this species may well be considered the type species.

The work of the writers on the present species, *Tetracotyle iturbei,* together with that on *Cercaria flabelliformis* and *Tetracotyle pipientis,* makes it possible to define the genus more carefully, without in the least deviating from de Filippi's original conception of the genus.

Redescription of Tetracotyle.—Holostome larva, oval, pyriform or ovate-oblong in contour, with ventral compression. Attachment apparatus consists of an oral sucker, ventral sucker (acetabulum) often degenerate and glandular, a ventral muscular genital pore usually somewhat larger than the acetabulum, and a pair of lateral suckers to the right and left of the pharynx, at times muscular, but also glandular in some species—all of these usually included within a muscular attachment cup. Primitive genital pore with or without functional connections with the genital organs. Excretory system having framework of an inconspicuous bladder, a pair of long cornuate vesicular trunks and a prominent transverse vessel which shifts its position in various species. Genital organs well differentiated in the larvae: consisting of a pair of oval testes, a pair of vitelline chorda, a median ovary and a posterior genital pouch. Nervous system highly modified. Parthenogenetic generations occurring in the mollusk, intermediate stage passed in vertebrates, and possibly in the case of *T. hirudinum* in leeches, and the definitive stage in higher vertebrates. Adult stage thot in all cases to be the genus Strigea.

The genus Tetracotyle is differentiated from Diplostomulum and Tyrodelphys by the presence of lateral grooves, which are primitively muscular, but at times glandular. These grooves may be situated at the anterolateral margin of the worm or may be ventrally placed. *Monocerca heterobranchi* Wedl has chitinous grooves at the anterior margins. It may represent a transition from the Tetracotyle to the Tylodelphys type. Were the internal anatomy of all the species better known, a more fundamental basis for classification would be afforded.

Synopsis of Described Species of Tetracotyle

1. *Tetracotyle* (*Distoma*) *crystallina* (Rud.) 1819

Outline oval; length 0.4 to 0.6 mm.; width 0.25 to 0.45 mm.; oral sucker 130μ in diameter; primitive genital pore 140μ in diameter, median ventral; acetabulum glandular, auriculate; lateral suckers with small spines, opening forward. Excretory bladder rhomboidal, canals meandering, branching anterior to primitive genital pore.

Encysted in muscles of Rana, Bufo, and Pelias (Viperus). Europe.

2. *Tetracotyle* (*Heptastomum*) *hirudinum* (Schomburgk) 1844

Outline pyriform; length 0.62 mm.; remainder of description quoted directly from Diesing (1858:370): "Acetabula quattuor limbo ciliata, ventralia maximum subcentrale, alterum minus postpositum versus marginem posticum, transverse elliptica, et duo multo minore longe elliptica parallela, cum acetabulo marginale in triangulum disposita." Since Schomburgk figured his fluke up-side-down, his "versus marginem posticum" means toward the anterior end, and "aperturae genitales discretae antrosum sitae" should read "aperturae genitales discretae posticum." Two oval or reniform testes are figured behind the ovary. The main excretory trunk is median, extending to the region just behind the testes at which place the transverse canal is

formed. The lateral canals are given off near the base of the main trunk; they give rise to many tubules and capillaries laterally disposed.

Recorded as parasitic externally, also in the genital organs of *Nephelis vulgaris* and *Clepsine complanata*. Europe.

3. *Tetracotyle percae-fluviatilis* Moulinié 1856

Outline oval; length 0.38 to 0.88 mm.; width 0.3 to 0.5 mm.; oral sucker 60μ; primitive genital pore 80 to 100μ, in the posterior half of the body; acetabulum small, inconspicuous; lateral suckers 66 by 133μ, lateral to pharynx. Crura long, meandering to posterior part of the body. Bladder small, lateral excretory trunks filiform, transverse vessel just behind primitive genital pore, secondary laterals from transverse vessel coursing forward.

Encysted in region of heart, *Perca fluviatilis*. Europe.

This species is credited to von Linstow in Lühe (1909: 170).

4. *Tetracotyle typica* Diesing 1858

Outline ovate to pyriform; length 10 mm.; width 0.62 mm.; oral sucker 59μ in diameter; primitive genital pore 79μ in diameter; acetabulum glandular, very large; lateral suckers auricular, subequal to oral sucker. Esophagus long, crura with many lateral ceca. Bladder hemispherical, pore subterminal, excretory stems meandering, branching in region of primitive genital pore; no transverse canal described.

Found in Lymnaea, Planorbis and Paludina in Europe; reported from *Lymnaea catascopium* and *Physa heterostropha* by Leidy for North America.

5. *Tetracotyle echinata* Diesing 1858

Outline oval; length 0.62 mm.; lateral suctorial grooves glandular, sparingly covered with spines 3 to 4μ long; grooves subequal to oral sucker. Network of excretory granules.

Encysted in oval capsules 0.5 to 0.6 mm. thick, in peritoneum of *Leuciscus idus* and *Acerina cernua*. Europe.

6. *Tetracotyle foetorii* von Linstow 1876.

Top-shaped, with transverse constriction anterior to primitive genital pore; length 1 mm.; width 0.48 mm.; oral sucker 130μ in diameter; primitive genital pore 170μ in diameter; acetabulum large, irregular, glandular; lateral suckers small, auricular. Crura from base of pharynx to region of acetabulum; large genital cell mass behind acetabulum.

Encysted in neck muscles of *Mustela* (*Foetorius*) *putorius*.. Europe.

7. *Tetracotyle colubri* von Linstow 1877

Anterior end elongate, posterior end elongate—cylindrical; few large spines with broad bases on surface of integument; length 0.54 mm.; width 0.3 mm.; oral sucker 78μ in diameter; primitive genital pore 120μ in diameter; acetabulum considerably larger than primitive genital pore; lateral suckers oval, lateral to oral sucker. Crura arising from base of pharynx, extending to posterior end of primitive genital pore.

In thick-walled capsules embedded in subcuticula, *Tripidonotus* (*Coluber*) *natrix* and *Pelias* (*Vulperus*) *berus*. Europe.

8. *Tetracotyle soricis* von Linstow 1877

Similar in most respects to *T. colubri*. In capsules 1.2 mm. by 0.54 mm.; oral sucker 66μ in diameter; primitive genital pore 110μ.

Embedded in connective tissue in a double capsule, *Sorex vulgaris*. Europe.

This description is inadequate to warrant the creation of this species, but future work on the species may show it to be well founded.

9. *Tetracotyle ovata* von Linstow 1877

Outline large oval; spines confined to suckers; length 0.84 mm.; width 0.57 mm.; oral sucker 98μ in diameter; primitive genital pore 130μ in diameter; acetabulum 160 to 210μ in diameter, opening backward ("larval anus" of von Linstow); lateral suckers elongate oval. Concentric rows of teeth on oral sucker and primitive genital pore.

Encysted in gut or peritoneum, or free capsules in body cavity, *Abramis (Blicca) bjoerkna, Osmerus eperlanus, Acerina cernua,* and *Abramis brama.* Europe.

10. *Tetracotyle lenticola* (von Linstow) 1878

Outline broadly pyriform; length 0.55 mm.; width 0.46 mm.; oral sucker 66μ in diameter; primitive genital pore 66μ in diameter; acetabuum about 60μ in diameter, with many radiating glands; lateral suckers at extreme anterolateral reaches, consisting of lenticular muscular grooves. Excretory bladder triangular, vesicular; lateral canals constricted, with racemose tubules thruout body. Digestive crura to region just anterior to excretory bladder.

In lens, *Abramis vimba.* Europe.

11. *Tetracotyle petromyzontis* Brown 1899

This species was first found by Müller in 1840 and described as a diplostome in the fourth brain ventricle of *Petromyzon fluviatilis.*

Synonymy:—Diplostomum of *Petromyzon fluviatilis* Müller 1840
Diplostomum petromyzi fluviatilis Diesing 1850
Tylodelphys petromyzontis fluviatilis Diesing 1858
Diplostomum mülleri Cobbold 1860.
Tylodelphys petromyci fluviatilis von Linstow 1878
Tetracotyle petromyzontis Brown 1899

Outline ovate, with oral end set off from body; length 0.42 mm.; oral sucker an ovoid cup; primitive genital pore slightly larger than oral sucker; acetabulum a longitudinal slit; lateral suckers auricular, multiglandular, just lateral to mouth. Pharynx powerful, ceca extending to subcaudal region. Genital cells consist of undifferentiated nuclear aggregates in region of primitive genital pore. Excretory bladder bicornuate, anterior tubules dendritic, posterior tubules prominently reflexed; transverse vessel split into two parts.

In fourth brain cavity of Ammocœtes. Europe.

Leydig arranged his species *Tylodelphys crainaria* with Henle's *T. rhachiaea* and Müller's Tetracotyle of *Petromyzon fluviatilis* since they possessed in common "calcareous granules" within the body tissues of the worms.

12. *Tetracotyle phoxini* nov. spec

Synonymy:—Tetracotyle from *Phoxinus laevis* Mataré 1910.

Outline pyriform, with constriction separating anterior and posterior parts of body; length 0.2 mm.; width 0.15 mm.; oral sucker and primitive genital pore subequal; acetabulum larger, midway between primitive genital pore and bladder; lateral suckers auricular lappets, to right and left of oral sucker. Pharynx well developed, embracing entire esophagus; ceca extending to acetabular region. Excretory bladder large, bicornuate; split longitudinal canals, with a transverse canal in region of primitive genital pore.

In brain and cranial cavity of *Phoxinus laevis.* Europe.

Mataré has brought together most of the true tetracotyles in his study, but he has also listed a number of diplostomula among these, as well as the agamodistoma of Wedl and of Leydig.

Explanation of Plate

a, acetabulum
ad, anterior dorsalis nerve
av, anterior ventralis nerve
c, cirrus pouch
ce, cecum
cy, cyst capsule
g, posterior genital pore
ge, genital nerve
in, integument
l, lateral sucker
lm, longitudinal muscle
o, ovary
p, pharynx
pc, modified parenchymaa cell
pd, posterior dorsalis nerve
pg, primitive genital pore
pv, posterior ventralis nerve
s, subesophageal commissure
t, testis
tr, transverse muscles
v, vagina

Fig. 1. Ventral view of *Tetracotyle iturbei*, × 126. 2. Frontal section of fluke thru digestive ceca, × 260. 3. Region of integument and subintegumentary tissues, × 1500. 4. Oblique frontal view of nervous system, × 396. 5. Sagittal section of fluke, showing connection of vagina with primitive genital pore and posterior genital pore, × 260. 6. Sagittal section of worm in region of pharynx, showing relation of retractor muscles to pharynx, × 396.

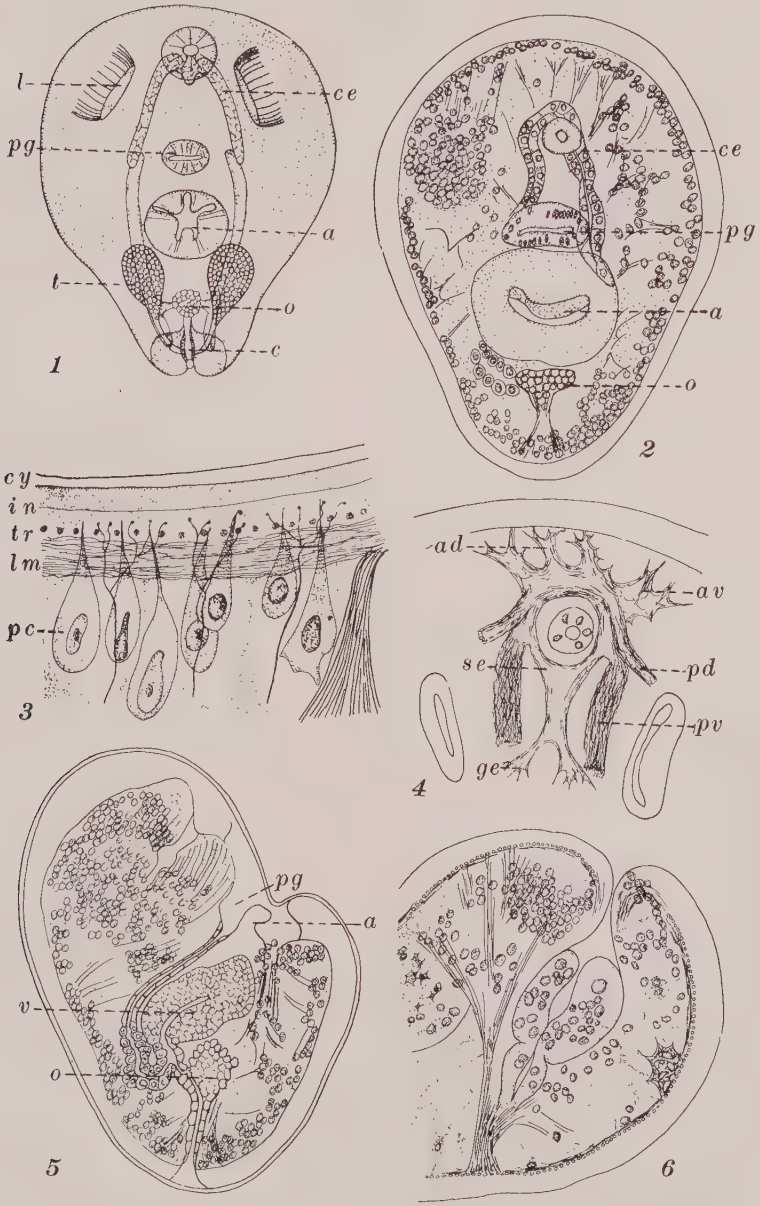

PLATE VI

13. *Cercaria* (*Tetracotyle*) *flabelliformis* Faust 1917

This is a tetracotyle in the pre-encysted stage. Its life history has been worked out thru the redia generation.

Outline ovate, with slight indication of caudal constriction; length 0.48 to 0.56 mm.; width 0.44 mm.; oral sucker 60μ in diameter; primitive genital pore 50μ in diameter; acetabulum confined to two transverse muscular lappets; lateral suckers oval in outline in young larva, wandering inward to sides of primitive genital pore and metamorphosing into lateral lappets in more mature larvae. Pharynx small; ceca sacculate, extending caudad two-thirds of body length. Excretory bladder inconspicuous; lateral canals with transverse vessel posterior to primitive genital pore; fan-shaped distribution of anterior tubules. Genital cell masses well defined, consisting of a club-shaped ovary, two vitellarian chorda, two testes posterior to ovary, and muscular genital cone.

In liver tissue, free or encysted, or in rediae, *Physa gyrina*. Corvallis, Montana.

14. *Tetracotyle pipientis* Faust 1918

Outline lyrate, with dense covering of spines; length 0.50 mm.; width 0.37 mm.; oral sucker 75μ in diameter; primitive genital pore 80μ in diameter, with a heavy crown of spines; acetabulum modified into a single transverse lappet; lateral suctorial organs elongate, obliquely placed, with large marginal spines. Pharynx small; ceca extending to center of primitive genital pore. Excretory bladder inconspicuous; lateral vessels, with transverse vessel far sephalad. Genital organs well defined, consisting of spherical ovary somewhat behind primitive genital pore, vitellaria in two diffuse chorda, two laterally disposed testes and ovoid genital cone.

Encysted in heavy capsules, mesentery and peritoneum, *Rana pipiens*, Chicago, Illinois.

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CONTRIBUCIÓN AL ESTUDIO DE LA PARASITOLOGIA EN VENEZUELA. ESTUDIO Y CLASIFICACIÓN DE UN DISTOMA

J. M. Romero Sierra
Jefe de los Trabajos Prácticos de la Cátedra de Anatomía Patológica

El día 1°. de mayo de este año, haciendo la autopsia del cadaver de un enformo procedente del Hospital Vargas, del servicio del Doctor M. A. Fonseca, mientras nos ocupábamos de buscar la ampolla de Vater para llevar a cabo la exploración del canal colédoco y del pancreático, siguiendo la técnica acostumbrada en la cátedra de Anatomía Patológica de esta ciudad, despues de habor abierto el duodeno entre dos pinzas y lavado con agua su mucosa, observamos en la superficie de esta membrana, al cabo de cierto tiempo de exploración con el estilete, un pequeño organismo que se movía, de aspecto al primer momento alargado, cilíndrico, de color oscuro, pero que en seguida pudo ser desplegado y mantenido extendido entre dos láminas de vidrio, apareciendo entonces con la forma de una hoja.

Resolví hacer el estudio y clasificación de este parásito, los cuales se encuentran expresados en las siguientes líneas.

DESCRIPCION

La observación del parásito me permite encontrar en él los siguientes caracteres:

Su cuerpo es de simetría bilateral. No está dividido en anillos, no es pues segmentado. No presenta miembros articulados. Es aplastado, delgado, desnudo, no recubierto de pestañas, foliáceo. En su contorno es más oscuro que el resto, excepto en un prolongamiento de él en forma de ángulo agudo en que no se nota ese tinte oscuro del contorno. Es ensanchado hacia el prolongamiento en forma de ángulo agudo de que he hablado y se estrecha para former este prolongamiento. El extremo opuesto a él que tiene la ventosa oral o bucal, de la cual hablaré más adelante, es redondeado o curvo. Es pues el conjunto del parásito semejant a una hoja: representando su prolongamiento angular, de que he hablado, el peciolo de esta hoja y el resto, el limbo; el cual resto tiene una forma de corazón.

Presenta dos ventosas: una ventosa oral o bucal y una ventosa ventral. La ventosa ventral está muy cerca de la ventosa oral. La ventosa bucal está en el vértice del prolongamiento del cuerpo en forma de ángulo agudo, del cual he hablado. La ventosa oral es más pequeña que la ventosa ventral. Las dos ventosas son redondeadas. La ventosa ventral tiene su abertura dirigida hacia la ventosa bucal.

Observo en seguida de la ventosa bucal un bulbo faringeano y un corto esófago. Este esófago es en forma de trapecio, sigue a dicho bulbo faringeano, menos teñido que dicha faringe, algo apagado; este trapecio tiene su pequeña base hacia el bulbo faringeano y su base mayor hacia la ventosa ventral; además este esófago es mucho más pequeño que el bulbo faringeano.

El tubo digestivo continúa el esófago, formando dos prolongamientos: uno que parte de un extremo de la base mayor del trapecio esofagiano y otro, del otro extremo de esta misma base. Estos prolongamientos se dirigen hacia el extremo del parásito opuesto a donde está situada la ventosa bucal; divergiendo (es decir alejándose el uno del otro) y guardando cada uno algún paralelismo con el borde que le queda más próximo de la extremidad del cuerpo del parásito en donde está la ventosa bucal; con dicha disposición continúan hasta llegar cada uno al lado y a alguna distancia de la ventosa ventral; entonces se inclinan algo hacia adentro; después a alguna distancia del sitio en que cambiaron de dirección, distancia que es próximamente igual a la que dista entre este sitio y su nacimiento en el esófago, estos dos prolongamientos divergen de nuevo hacia afuera, se alejan el uno del otro hasta terminarse cada uno en ramificaciones, que a su vez se ramifican. En el trayecto, desde que parten del trapecio esofagiano hasta que se resuelve en sus ramificaciones terminales, cada prolongamiento intestinal presenta ramas. En la porción de cada prolongamiento que guarda algún paralelismo con el borde que le queda más próximo de la extremidad del cuerpo del parásito donde está la ventosa bucal, hasta el sitio donde cambian de dirección al lado y a alguna distancia de la ventosa ventral, observo solo ramas que se dirigen hacia afuera.

Dimensiones tomadas después de hecha la preparación: Largo: Om., 0125. Mayor anchura: 0-, 007. Distancia entre la ventosa bucal y la ventosa ventral: Om., 0015. Pero parece haber sufrido algo de detracción.

Alrededor de la ventosa ventral, menos de la parte de este órgano que está hacia el esófago, se agrupan una gran cantidad de elementos de forma oval. Ya los otros caracteres que he observado y que he ya relatado me habían permitido hacer, por el estudio comparativo, la clasificación de este parásito; la cual se verá más adelante; y pude, por consiguiente, al observar estos elementos ovales, y mediante el mismo estudio, reconocer en ellos, por su forma y situación, los huevos del parásito.

CLASIFICACION

Por todos los caracteres que he observado en este parásito y su estudio comparativo me parece que puede decirse que pertenece a la ramificación de los Gusanos, clase de los Platelmintos, orden de los

Trematodes, sub-orden de los Distomianos, familia *Distomidados,* género *Fasciola,* especie *hepatica.*

Fasciola hepatica tiene la siguiente "Sinonimia: Gran Distoma.—*Distomum hepaticum* Retzius 1786.—*Fasciola humana* Gmelin 1789.—*Distomum caviae* Sonsino 1890.—*Cladocoelium hepaticum* Stossich 1892."

"Este Gusano vive en los canales biliares del Carnero y del Buey; se lo ha igualmente observado, pero más raramente, en el Búfalo, la Cabra, el Camello, la Llama, el Caballo, et Asno, el Marrano, el Conejo doméstico (Railliet), el Conejo de conejar, la Liebre, el *Cobaye* (Sonsino) y en el Hombre" (Brumpt, 1913).

"Anatomía patológica.—Los Distomas irritan los canales biliares y producen una atrofia del tejido hepático; los canales biliares esclerosados hacen salida en la superficie del órgano y resaltan por su color blanco sobre el fondo rojo castaño del hígado...

Las lesiones histológicas son muy interesantes, el epitelio de los canales biliares prolifera y da nacimiento a adenomas biliares de un gran espesor. La pared es fuertemente esclerosada y la eosinofilia local en general muy marcada.

Distribución geográfica.—En Europa, *Fasciola hepatica* puede existir y aclimatarse por todas partes donde se encuentra *Limnaea truncatula* Müll. (*L. minuta* Drap.) que le sirve de huésped intermediario. Este Molusco es repartido en toda la Europa, el Thibet, el Asia Menor y el territorio del Amour (R. Blanchard). En la América del Sur, donde el Distoma es bastante repartido, su huésped intermediario es *Limnaea viator* de Orb.; en la América del Norte, este huésped es *L. humilis* Say; en las islas Sandwich: *L. oahuensis* Souleyet, y *L. rubella* Lea (Verdun). Es probable que el gran Distoma posee igualmente la posibilidad de evolucionar en otras especies de Limneas exóticas.

Papel patógeno.—El gran Distoma es un parásito raro y enteramente accidental en el Hombre, que ciertamente no le ofrece buenas condiciones para continuar su evolución. En los animales domésticos, este Gusano ocasiona una anemia perniciosa, conocida de los veterinarios bajo el nombre de *podredumbre* o de *caquexia acuosa,* de la cual el diagnóstico se hace fácilmente por la investigación de los huevos en las materias fecales (Brumpt, 1913).

Brumpt, a quien cito actualmente, habla también del diagnóstico por el precipito-diagnóstico y la fijación del complemento.

"El gran Distoma no ha estado observado que una veintena de veces en el Hombre: en el hígado, en la sangre, en el pulmón y en los abcesos sub-cutáneos. . . ."

Se dice que los jóvenes Distomas, fijándose en la cavidad bucal y en la faringe, producen una enfermedad llamada *Distomatosis bucofaringea.* Brumpt, refiriéndose a esta enfermedad, dice: "Dos jóvenes

Perros nutridos durante más de un mes de hígados de Carnero encerrando millares de jóvenes *Fasciola* y *Dicrocœlium* no me han jamás mostrado ningún parásito fijado sobre la mucosa bucal. Yo creo pues que sería necessario estudiar de nuevo la etiología de esta curiosa enfermedad, pues el papel de los Distomas me parece bien dudoso."

Verdun, refiriéndose a la Fasciola hepatica, dice: "En el Hombre, la distomatosis hepática, debida a esta especie, es más bien rara, pero se muestra siempre como una afección grave" (Verdun, 1913).

LA AUTOPSIA

Datos previos.—Cadaver N°. 918. Procedente del Hospital Vargas. Sericio del Doctor M. A. Fonseca. Sala N°. 9. Cama N°. 10. Diagnóstico clínico: Parasitosis intestinal. Hora del fallecimiento: las 9 a. m. del día 1°. del presente mes. Entró a la Escuela de Medicina el 1°. de mayo de 1918, a las 10 y media a. m. Nombre R. D.

Resumen del protocolo de la autopsia, el cual es el N°. 288.—Aspecto: enflaquecido. Sexo: masculino. Edad aproximada: 40 a 50 años. Tamaño: lm., 65. Pericardio: con derrame. Corazón: normal, tiene 225 gramos de peso. Pulmones: el izquierdo adherente, antracósico, se nota en varias partes zonas de endurecimiento, congestionado, pesa 515 gramos; el derecho es adherente, con cavernas en la base y muy congestionado, pesa 530 gramos. Ascitis. El diafragma es descendido a la izquierda y a la derecha. Gran epiplón: retraido. Bazo: pequeño, cápsula arrugada, pesa 60 gramos. Riñón derecho pesa 140 gramos. Hígado: con adherencias en la cara superior, pesa 1300 gramos. Se encontró en el duodeno el Distoma que he estudiado y clasificado y el *Ankylostoma americanum* o *Necator americanus.*

Al preparar éste trabajo, he leido las siguientes importantes publicaciones nacionales, referentes a Distoma y Distomatosis: Estado actual de la Parasitología en Venezuela por el Doctor Jesús Rafael Rísquez, Distoma y distomatosis en Venezuela por el mismo autor, Sobre Distomatosis Hepáticas en Venezuela por el Doctor Horacio Bello y Revisión de nuestras (?) Distomatosis hepáticas por el Doctor J. B. Ascanio Rodriguez. Pero no he tenido noticia de que en Venezuela se haya encontrado otra vez en la autopsia este Distoma a que me refiero, en el estado adulto, en el organismo humano.

Caracas, 19 de mayo de 1918.

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FLIES OF THE GENUS DROSOPHILA AS POSSIBLE DISEASE CARRIERS

A. H. Sturtevant
Columbia University

It was pointed out by Howard (1900) that the habits of certain species of Drosophila are such as to make them possible carriers of typhoid fever or other diseases. It is the purpose of the present paper to record certain observations bearing on this possibility.

Drosophila melanogaster Meigen (*ampelophila* Loew).*—This cosmopolitan species was bred from human excrement by Howard, and there are a few other such breeding records from tropical regions; but I am very doubtful of the specific determinations in the latter cases. My own observations in the American tropics indicate that *D. melanogaster* is there extremely rare as an excrement fly, while *D. caribbea* (see below), which resembles it very closely, is common about excrement. Howard's breeding record almost certainly rests on a correct specific determination; and, this being the case, the habits of the adult flies are such as to make them open to suspicion, for *D. melanogaster* is always common about unprotected fruit in grocery stores and houses. Nevertheless, the species is a decided rarity about excrement, usually breeding in decaying fruit, and so is probably not an efficient disease carrier.

Drosophila caribbea Sturtevant.—This species, common throughout the American tropics, has habits very similar to those of *D. melanogaster,* both in larval and in adult life, but is much more frequently attracted to excrement. In Panama I have found it not uncommon about such material; and in Havana, Cuba, Mr. J. R. Taylor, of Las Animas Hospital, showed me specimens bred from the feces of a dysentery patient.

Drosophila busckii Coquillett, and *D. funebris* Fabricius.—These two species, both widely distributed and probably cosmopolitan, were both recorded by Howard as caught on human excrement. It seems probable from their habits that they would breed on such material; but they are not likely to be important as disease carriers, since they are not common about food. *D. busckii* frequently breeds on potatoes and other foodstuffs, but not until they are seriously decayed.

* The writer has in press (Bull. Amer. Mus. Nat. Hist.) a synopsis of the Nearctic species of Drosophila, containing keys that include all the species of the genus known from the United States.

D. repleta Wollaston.—This species is common from Massachusetts and Indiana, south to Brazil, and also occurs in the Old World. Unlike many other species of the genus, it is most frequent near houses. It is attracted to various organic substances, and it has the peculiar habit of coming to rest frequently on a white surface. In the eastern states the form is most easily collected about urinals that are not kept clean or thoroughly disinfected — such places as are frequently to be found in saloons or railway stations. The next most likely place to find the species is in kitchens or restaurants, especially on bread or on white walls or tablecloths. I have seen it frequently both in restaurants and in urinals in Boston, New York, Washington and elsewhere.

In Cuba *D. repleta* literally swarms around any place where excrement is allowed to remain in quantity. It is by far the commonest fly in such places, as Dr. C. W. Metz and I have observed at Guines, Aguada Pasajeros and elsewhere. Isolated deposits are not favorable, being usually attacked chiefly by species of Leptocera (Limosina) and Sepsis — which forms are practically never found about food and are therefore not dangerous.

D. repleta has a wide range of breeding habits, so that control measures would be difficult. It breeds on various kinds of fruit (banana, pineapple, tomato, etc.), though it is not so common on fruit as are several other species of the genus. It will also breed on decayed potatoes, flour paste, moist bran, and various similar substances. Although it has not been bred from excrement, there can be little doubt that it does use such material for larval food.

The literature would lead one to suspect *Drosophila melanogaster* as the most dangerous species, with *D. funebris* and *D. busckii* of doubtful significance; but more detailed observations lead to the conclusion that none of these three species can be particularly dangerous, whereas *D. repleta*, and *D. caribbea* in the tropics have habits of such a sort as to make them important as possible disease carriers.

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A NEW CERCARIAEUM FROM NORTH AMERICA*

WILLIAM W. CORT
University of California

During July and August, 1915, while studying at the University of Michigan Biological Station at Douglas Lake, Michigan, rediae containing tailless cercariae were found in the livers of nine out of twenty-six specimens examined of *Planorbis campanulatus smithii* Baker. Since the adult of this species of cercaria is not known, I will place it in the provisional generic group Cercariaeum and give it the name *Cercariaeum mutabile.*

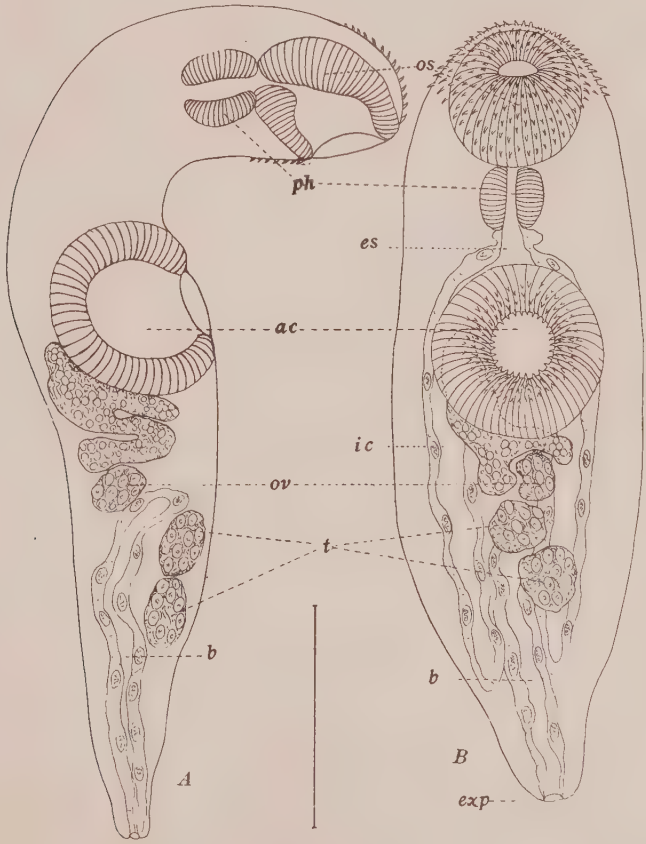
The hosts were collected from shallow water along the shore of the lake. The rediae (Figs. 1 and 2) filled the liver of the infected host and contained cercariae in all stages of development. The smaller rediae were quite mobile, altho they had no locomotor appendages. Since the rediae were without birth pores, the cercariae were obtained for study by breaking them open. The digestive sac of the redia (*ds*) is short, reaching even in the younger specimens to less than a third of the total length. The excretory system of the redia is divided into two entirely distinct halves. From the excretory pores (*exp*) short bladder tubes (*bt*) run forward which bifurcate into collecting tubes (*ct*) extending forward and backward. The anterior collecting tubes receive capillaries (*c*) from three flame cells (*f*) on each side, while the posterior collecting tubes receive the capillaries from two flame cells. The excretory system of this redia was very difficult to work out and I am not sure that all the flame cells present were located.

Cercariaeum (*mutabile*) (Text-figure A and B) is a large form with the adult characters well developed and almost no adaptive larval characters. No trace of a tail could be found at any stage of development of the cercaria. This cercaria is very mobile, being able to extend and contract its body to a remarkable extent. At greatest extension the body becomes so long and narrow that it resembles a nematode except for the large acetabulum which juts out prominently. When at greatest contraction the body is almost round and the acetabulum is pulled up against the oral sucker. The cercaria moves actively on a substratum by the use of its suckers, but is unable to swim.

The suckers of *Cercariaeum mutabile* are large and powerful. The acetabulum is the larger, having a ratio to the oral sucker of about three to two. The cuticular spines (Text figure B) cover a very limited area of the anterior tip. They are also found in several rows

* Publication .... from the University of Michigan Biological Station.

surrounding the opening of the acetabulum. The digestive system consists of a large muscular pharynx (*ph*), a short esophagus (*es*) and intestinal ceca (*c*), which reach almost to the posterior end of the body.



Cercariaeum mutabile

A, side view; B, ventral view; *os,* oral sucker; *ph,* pharynx; *ac,* acetabulum; *ov,* ovary; *t,* testes; *b,* excretory bladder; *es,* esophagus; *ic,* intestinal cecum; *exp,* excretory pore. Scale equals 0.1 mm.

The excretory system of *Cercariaeum mutabile* (Fig. 3) consists of a single club-shaped bladder, a complicated series of collecting tubes and sixty-four flame cells with their capillaries, arranged in eight groups of four on each side. On the left side (Fig. 3) the flame cells and their capillaries are not shown. The figure is drawn from the dorsal side and the accessory collecting tubes, the capillaries and flame cells which supply the ventral side are shown in dotted lines. The principal collecting tubes on each side divide each into two tubes, the

posterior (a') of which is much longer than the anterior. The subdivisions of these two tubes (a and a') correspond except that the relations are reversed. It can be seen that of the subdivisions of a which I have designated b and c, the one which extends posteriorly (b) does not further subdivide, while of the subdivisions of a' which are designated b' and c' it is the one which extends toward the anterior end (b') which does not again subdivide. This same relation is carried out in the third subdivision (cf, d and d' and e and e'). The capillaries from the flame cells join the accessory collecting tubes in definite groups of four, half of which are dorsal and half ventral. The flame cells are so distributed that every region of the body is drained. The extent of the subdivisions of the collecting tubes, the large number of flame cells and the definite arrangement of the capillaries into groups suggest that the excretory system of this cercaria is fully developed and represents the adult condition for the species.

The excretory system of *Allocreadium isoporum* (Looss) described by Looss (1894: 51-52, pl. 5, fig. 103) resembles in certain striking particulars the system just described for *Cercariaeum mutabile*. In *Allocreadium isoporum* the number of flame cells in each capillary group is four and the character of the bladder and the position of the main collecting tubes is the same as in my species. There are differences in the total number of capillary groups of which there are only six on each side in *Allocreadium isoporum*, and also in the arrangement of the accessory collecting tubes. The fundamental resemblances between the excretory systems of these two species must in my opinion indicate some degree of relationship.

The reproductive system of *Cercariaeum mutabile* is so far along in development that the adult arrangement of the organs can be partially made out (Text figure A). The testes (t) are located diagonally one behind the other along the longitudinal axis of the body about the middle of the post-acetabular region. They are ventral in position while the ovary (ov) which is just in front of them is near the dorsal surface. I was unable to clearly define the outlines of the other reproductive organs or to be certain of the location of the genital pore.

DISCUSSION

Since the provisional genus Cercariaeum is based on the single character of the absence of a tail in the fully developed cercaria within the sporocyst or redia, it is evidently not a natural group. The loss of the tail would seem to be due to the degeneration of this organ following the adoption of a type of life history in which the free swimming state is omitted. Such an adaptation might arise in any group of digenetic trematodes. Species which may be correctly placed in this provisional genus should be carefully distinguished from free

agamodistomes, which are larval distomes which have escaped from their sporocysts or redia and are waiting unencysted in secondary intermediate hosts to be carried into their final hosts. Sometimes a cercaria becomes an agamodistome in the same host which harbors its sporocyst or redia. This is the case with the so-called *Cercariaeum helicis* (Leidy), found in species of genus Helix. Hofmann (1899) finds that the cercaria of this species develops in sporocysts in the tissues of the snail host. This cercaria, which has a very degenerate tail, escapes from the sporocyst and migrates into the kidney of the same snail where it lives as a free agamodistome until it is carried passively into its final host. Such a life history shows the free life of the cercaria reduced to a passage from one organ of the snail to another. This life history approaches the condition found in the Cercariaeum group in which the free stage is very probably entirely omitted from the life history.

Two species of the provisional genus *Cercariaeum*, *Cercariaeum limnaei obscuri* Ercolani and *Cercariaeum paludinae impurae* Filippi (see Lühe, 1909, 208) resemble *Cercariaeum mutabile*. Lühe (1909, 93) refers the second of these cercariae to the species *Asymphylodera tincae* and suggests that the other belongs to some Asymphylodera species. The structure of these forms is not fully enough described to make a detailed comparison possible. *Cercariaeum mutabile* differs from *Cercariaeum paludinae impurae* in spination, in the size of the digestive sac of the redia and in the length of the esophagus of the cercaria. Further its structure is very different from that of the members of the genus Asymphylodera which have only one testis and a very small round excretory bladder.

Cercariaeum mutabile in contrast with such types of larval trematodes as the schistosome or stylet cercariae shows a considerable development of adult structures and practically no adaptive larval characters. The contrast is very striking between this cercaria and such a form as the cercaria of *S. japonicum* (Cort, 1918) in which adaptations for penetration dominate the whole structure, and adult characters are practically undeveloped. Since *Cercariaeum mutabile* has no adaptations for swimming, encystment or penetration, it seems very probable that there is no free swimming period in its development, and that it must be carried passively into some final host which feeds upon the snail intermediate host.

Altho I have no direct evidence in regard to the further development of my Cercariaeum, structural comparisons give some clue to its relationship. As stated above the similiarity of the excretory system of *Cercariaeum mutabile* to that of *Allocreadium isoporum* (Looss)

Explanation of Plate

Cercariaeum mutabile. Scale equals 0.1 mm.

Fig. 1. Redia showing contained cercariae; *ph,* pharynx; *ds,* digestive sac; *c,* fully developed cercaria; *uc,* undeveloped cercaria.

Fig. 2. Redia showing the excretory system; *exp,* excretory pore; *bt,* bladder tube; *ct,* collecting tube; *c,* capillary; *f,* flame cell.

Fig. 3. Excretory system, dorsal view. On the right side of the figure all parts of the excretory system are shown but on the left side the capillaries and flame cells are omitted. Anterior subdivisions of the collecting tube on the left side of the figure are labeled *a-f* and the corresponding posterior subdivisions *a'-f'*. Accessory collecting tubes, capillaries and flame cells of the ventral side are shown with dotted lines. Letters as in text figure; also, *mct,* main collecting tube; *act,* accessory collecting tube.

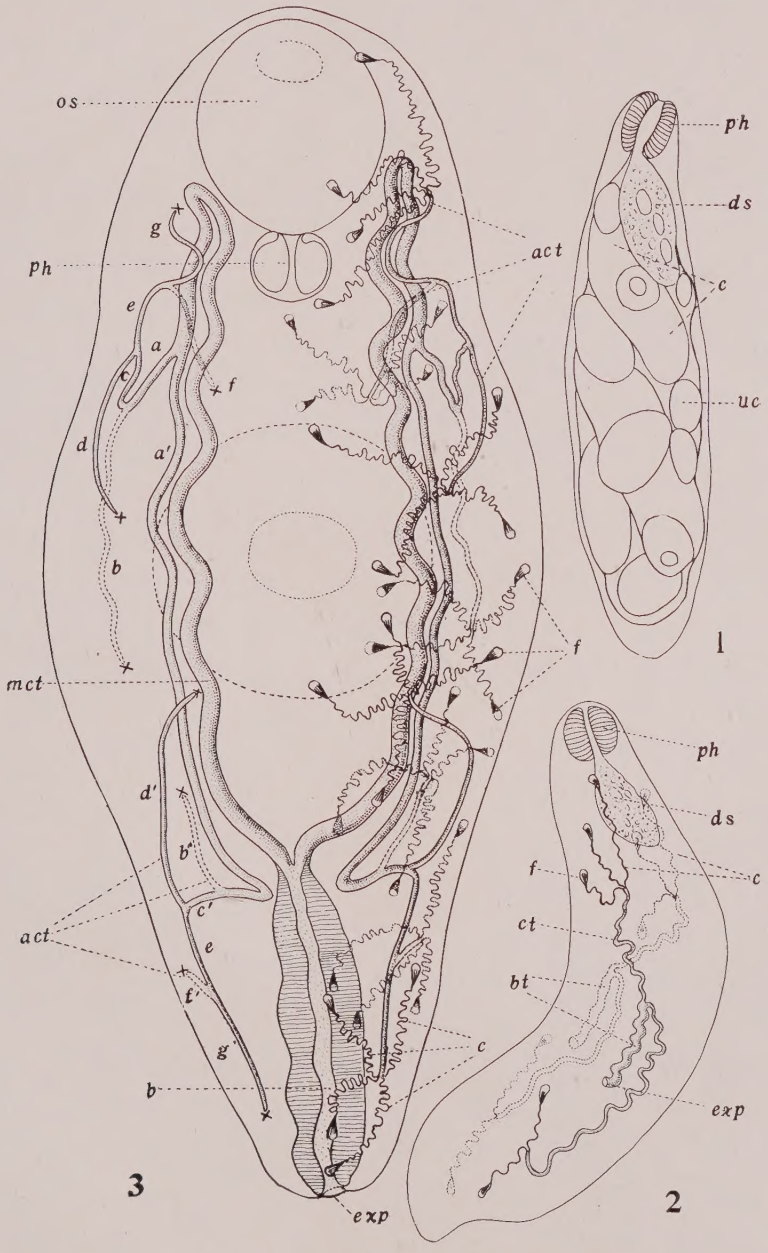

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PLATE VII

seems to indicate relationship. Further, the large size of the ventral sucker of my species and the characteristics of its digestive and reproductive systems place it in the subfamily Allocreadiinae Odhner, or at least very close to this group.

SUMMARY

Cercariaeum mutabile is a new species described from *Planorbis campanulatus smithii* from Douglas Lake, Michigan.

This cercaria has practically no adaptive larval characters and a considerable development of adult characters, evidently correlated with the omission of the free swimming stage from its life history.

The excretory system consisting of a simple club-shaped bladder, a series of collecting tubes, and sixty-four flame cells with their capillaries arranged in eight groups of four on each side.

The adult of *Cercariaeum mutabile* is not known, but its structure relates it to the subfamily Allocreadiinae Odhner.

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REVIEWS AND NOTES

The recent receipt of the initial number, July, 1918, in the sixth volume of the Indian Journal of Medical Research leads naturally to a review of this periodical which has done notable work in the field of parasitology. Founded in July, 1913, as the official organ of the Indian Research Fund and devoted entirely to the publication of research work directly or indirectly connected with medical and sanitary science, it has published each year since four numbers of goodly size that are marked no less by the high character of the papers contained than by the attractive appearance and admirable illustrations they present.

From the start marked emphasis has been laid on parasitology by the amount of the material published in that field and in the first number half of the papers or more belong in that category. It would be impracticable here even to mention all the articles on various phases of medical zoology which have been printed in the first five volumes. But their varied character may be rightly estimated from the fact that the last (fifth) volume includes papers on the life cycle of *Schistosoma spindalis,* on atypical malarial parasites, Negri bodies, Kala-azar, entamebic cysts, *Ochromyia jejuna,* parasitic Muscidae, ancylostomes, spirochaetes, Trichomastix, mosquitoes, and others on insects as well as on topics less immediately related to the subject but no less interesting to the parasitologist. The regular and normal appearance of so extensive and valuable a series during the height of a world war demonstrates indubitably the permanence of the foundations on which it rests.

The Indian Research Fund Association is to be congratulated on having established and maintained a publication of such high rank among the medical scientific journals of the world. To investigators in parasitology it has become indispensible, and one cannot doubt that it has furnished both at home and abroad a real stimulus to the development of the subject that will show itself in an ever widening circle of workers and in a constantly growing series of contributions of importance.

Professor Raphael Blanchard, editor of the Archives de Parasitologie, which was printed at Lillie and has been suspended since 1914, desires an announcement made of the fact that when Lillie recently passed into the control of the French, part 4 of volume 16 of the Archives, completely printed and dated August 1, 1914, and the seven first signatures of volume 17, plates, cuts, manuscripts, etc., were found uninjured.

Part 4 of volume 16 will be distributed immediately and the Archives will again make its appearance regularly as soon as it is possible to establish conditions for its appearance.

Professor Blanchard's new address is 4, Avenue du Président Wilson, Paris, 8e.

Sanidad y Beneficencia, Boletin Oficial, of Havana, Cuba, has published as a double number a splendid memorial to Dr. Carlos J. Finlay. The number contains as frontispiece a portrait of this distinguished student of medicine and parasitology and includes some twenty articles on his work and the recognition it has received in various ways.